HYDROLYSIS OF PEPTIDE BONDS

PARTICIPATION OF A CARBOXYL GROUP IN AMIDE HYDROLYSIS

A thesis presented for the
degree of Doctor of Philosophy in Chemistry
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by

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CONTENTS

ABSTRACT

In order to assess the effect of substituents on the participation of a neighbouring carboxyl group in the hydrolysis of amides, seventeen substituted succinamic acids have been prepared. Their rates of hydrolysis have been determined at three temperatures, and their rates and thermodynamic functions have been correlated at 70°. The mechanism of the hydrolysis has been discussed and the effect of the substituents interpreted in terms of their bulk and electronic characters.

INTRODUCTION

The hydrolysis of carboxylic acid derivatives has become one of the most extensively investigated fields of organic chemistry. As early as 1792 Scheele[1] studied the catalytic effect of dilute acids and alkali on the hydrolysis of esters. Since that time continued studies have resulted in the accumulation of a large body of data regarding the effect of structure on the reactivity in these systems. This information, together with information gained from tracer and exchange studies, has been reviewed[2], and has been of importance in determining the mechanism of the hydrolysis of carboxylic acid derivatives.

The field of catalysis of these reactions has received new impetus from the recent rapid advances made in the elucidation of the mechanisms of enzymatic hydrolysis. The similarity of intramolecular catalysis and enzymatic catalysis[3] has resulted in the construction of model systems which contain functional groups similar to those found at the "reactive site" of the enzyme. One such functional group is a free carboxyl group.[4]

The effects of anchimeric assistance by neighbouring carboxyl groups have been known for some

time. The rapid hydrolysis of some β-cyano-acids was observed as early as 1889[5]. Measurements on the rates of spontaneous hydrolysis of β-thiocyanatopropionic acid by Fredga[6] have shown that the undissociated carboxyl group is involved in the hydrolysis since the anion is quite stable in aqueous solution.

Wideqvist[7] has investigated the stability of a range of cyano-acids in water and shown that only β-cyano-acids are spontaneously hydrolysed. He concluded that this ease of hydrolysis of the cyano-acid depended on the direct interaction of the cyano group and the carboxyl group via a cyclic mechanism involving hydrogen bonding. He also observed that the resulting succinamic acids were subject to spontaneous hydrolysis in aqueous solutions.

Further evidence of intramolecular catalysis in the hydrolysis of amides is found in the acid catalysed hydrolysis of proteins[8,9] where the peptide bonds on either side of aspartic acid residues are particularly susceptible to mild acid hydrolysis. Partridge and Davis[8] suggested that the undissociated β-carboxyl group acted as a local source of protons, but the involvement of the carboxyl group was not demonstrated until Leach and Lindley[10] showed that N-substituted asparagines were only hydrolysed as the undissociated carboxylic acid.

Intramolecular participation of the carboxyl group has also been observed in the hydrolysis of esters. The phenomenon was first recognised by Garrett[11] in the studies by Edwards[12] on the solvolysis of acetylsalicylate. Garrett[13] greatly extended these studies to include a number of substituted acyl salicylates. The pH-rate profiles of these solvolyses indicated hydronium and hydroxide ion catalysis at low and high pH respectively, and catalysis due to the participation of the carboxylate anion in the range pH 4-8.

The hydrolysis of methyl hydrogen phthalate has been reported by Bender[14] to involve the participation of the carboxylate anion. In contrast to these findings it has been reported that the undissociated carboxyl group is the reactive species in the hydrolysis of methyl hydrogen 2,3-di-(t-butyl)succinate,[15] methyl hydrogen 3,6-dimethylphthalate,[16] ethyl hydrogen maleate,[16] and ethyl hydrogen citraconate.[16]

Bruice and Thanassi[17] were unable to duplicate the work of Bender. They found that the rate of hydrolysis of methyl hydrogen phthalate was proportional to the concentration of the undissociated acid. Further studies showed that the mechanism and the rate of solvolysis of phthalate monoesters depended upon the pK_a of the conjugate

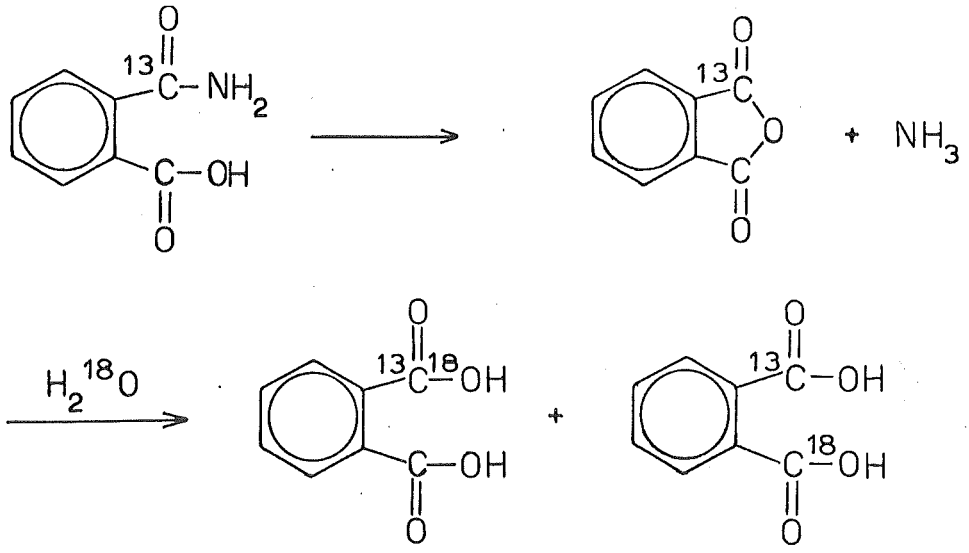
acid of the leaving group.

Table I[17]

Order of increasing rate constant for the hydrolysis of phthalate monoesters.	pK_a
$-OCH_3$	15.5
$-OCH_2-CH_2Cl$	14.3
$-OCH_2-C\equiv CH$	13.6
$-OCH_2-CF_3$	12.4
$-OC_6H_5$	10.0

The change in mechanism from $-CO_2^{\ominus}$ to $-CO_2H$ participation for the hydrolysis of phthalate monoesters occurs when the alcohol from which the ester is derived has a pK_a of ca. 13.6.

The formation of an intermediate cyclic anhydride in these reactions was first demonstrated by Bender.[18] He obtained indirect evidence for the existence of the anhydride from a tracer experiment involving the hydrolysis of phthalamic acid-carbox-amide $[^{13}C]$ in $H_2{}^{18}O$.



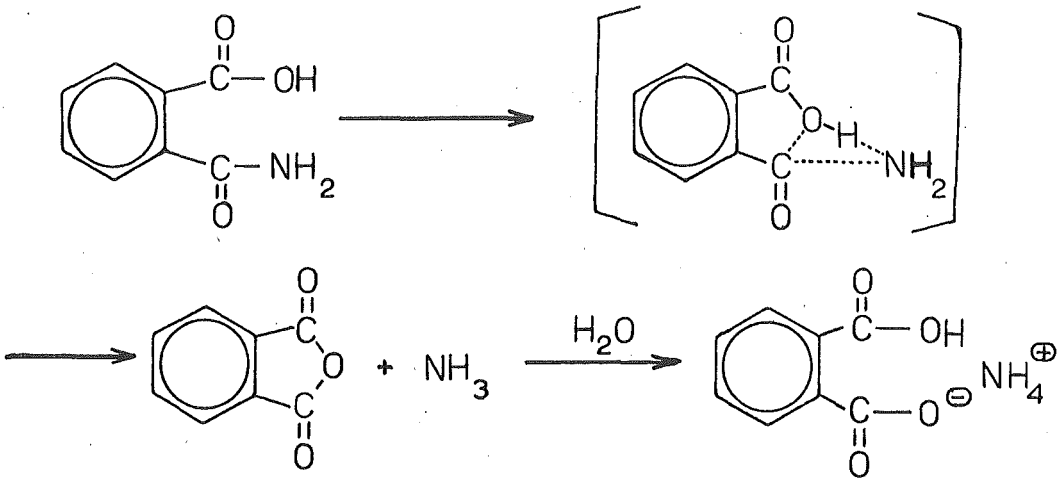
A similar experiment involving the intermolecularly catalysed hydrolysis of p-nitrophenyl benzoate by $\left[{}^{18}O_2\right]$ acetate ions indicated that acetic-benzoic anhydride was formed in the course of the hydrolysis. This was interpreted, by Bender,[19] as evidence for the formation of the corresponding cyclic anhydride in the intramolecularly catalysed hydrolysis of esters by a carboxylate anion. Further evidence of the existence of the anhydride was supplied by Bruice and Pandit.[20] They showed that the intermediate in the hydrolysis of the mono-p-methoxyphenyl esters of maleic and 5,6-endoxo-Δ^4-tetrahydrophthalic acids hydrolysed at identical rates to those of their anhydrides.

Eberson has demonstrated that methyl hydrogen 2,3-di-(t-butyl)succinate,[15] and methyl hydrogen 3,6-dimethylphthalate[16] are not hydrolysed at low pH in spite of the elimination of methanol, as the intermediate anhydrides are stable in aqueous solution and may be isolated. He[21] has also shown that the substituted succinic acids cyclize in their undissociated form in aqueous solution to give their anhydrides.

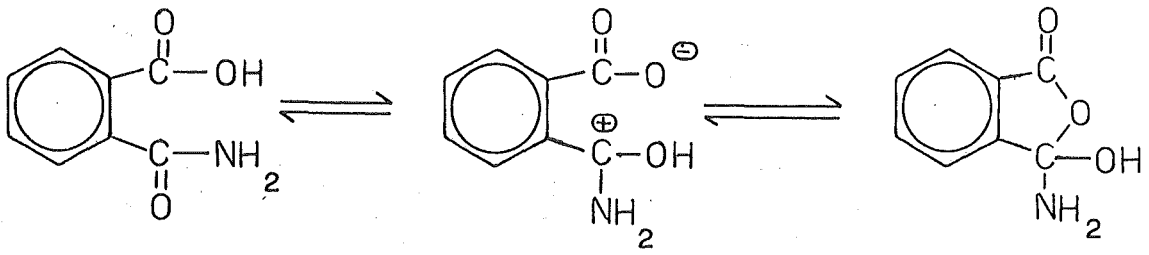
All these facts fit the mechanisms proposed by Bender.[18]

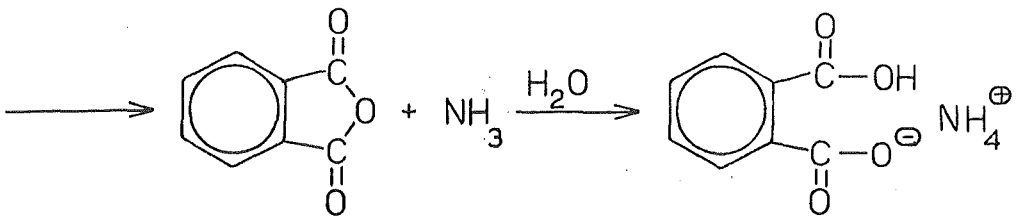
Carboxyl Group Participation

Mechanism I

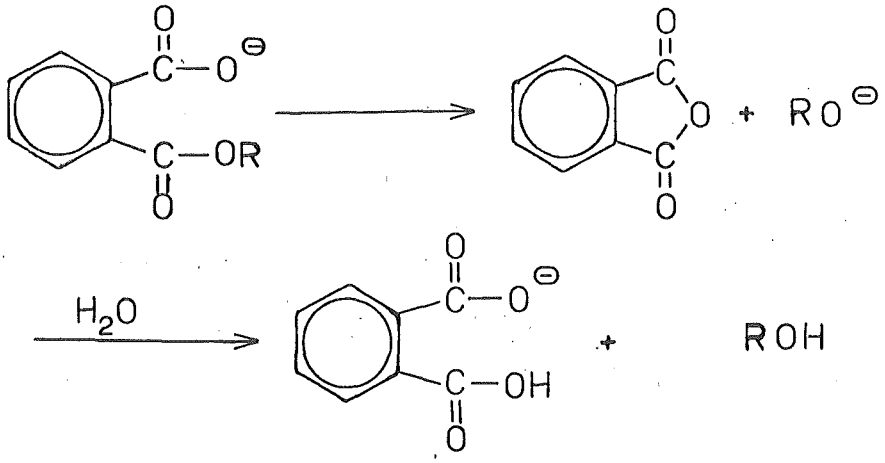


Mechanism II





Carboxylate Anion Participation



The mechanisms I and II are kinetically indistinguishable and remain largely a matter of choice.

The effect of substituents on the intramolecular participation of the carboxylate anion in the hydrolysis of the monoesters of glutaric and succinic acids has been examined by Bruice and Pandit,[20] and

Bruice and Bradbury.[22] The increase in the rate of hydrolysis with substitution was attributed by them to steric effects which reduce the nonprofitable rotamer distribution in the ground state.

In the work described in this thesis a number of substituted succinamic acids have been prepared and their rates of hydrolysis measured. Both the electronic and steric properties of the substituents have been found to be responsible for the variations in the rates of the intramolecularly catalysed hydrolysis.

EXPERIMENTAL

PREPARATION OF THE SUCCINAMIC ACIDS

General

Melting-points and boiling-points are uncorrected. Reference melting points are given in parentheses after the measured figures.

Microanalysis were carried out at the Microanalytical Laboratory, University of Otago.

All compounds containing asymmetric carbon atoms are racemic mixtures unless otherwise indicated.

The term "dried" implies that the organic phase was dried over magnesium sulphate unless another drying agent is specified.

OUTLINE OF PREPARATIVE METHODS

The amic-acids prepared during the course of this work can be divided into two main groups according to their parent acids. They are the (a) amic-acids of asymmetrically substituted succinic acids, and (b) amic-acids of symmetrically substituted succinic acids.

(a) Amic-acids of Asymmetrically Substituted Succinic Acids

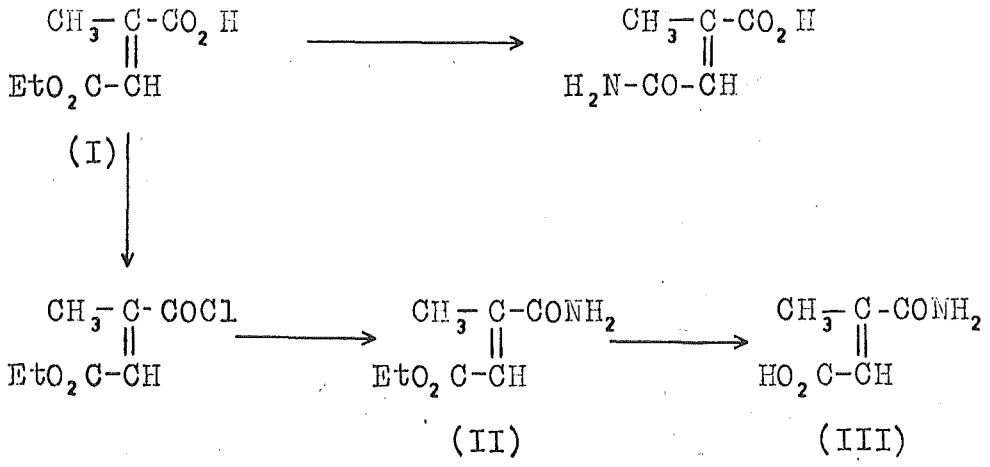
The preparation of succinamic acids belonging

to this group is complicated by the fact that each parent acid gives rise to two isomeric amic-acids. A number of different synthetic routes are available which will give selectively only one isomer.

(i) Hydrogenation of Substituted Fumaric Acids

One of the earliest methods used is the hydrogenation of substituted fumaric acids. Anschutz[23] found that the partial hydrolysis of the diesters of mesaconic acid selectively gave the α-ester (I). From this compound he was able to prepare both of the isomeric mesaconamic acids according to the scheme shown in Fig. I. The corresponding methylsuccinamic acids are obtained by hydrogenation.

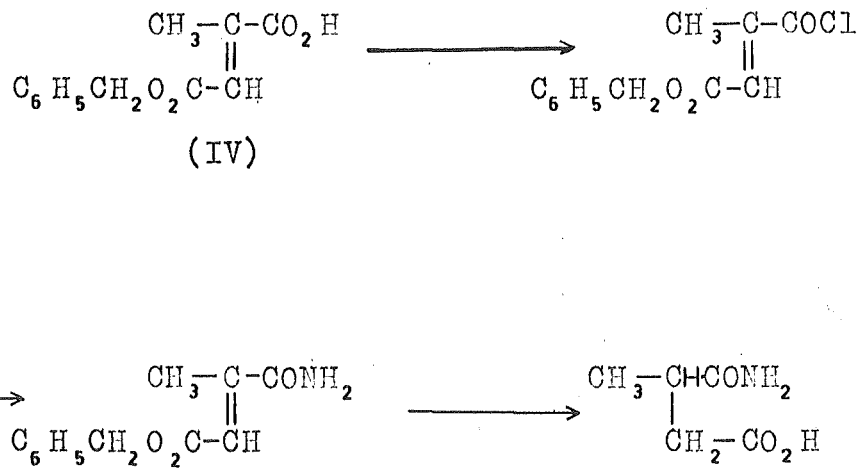
Fig. I



When Cocker and Fateen[24] tried to repeat Anschutz's preparation of β-mesaconamic acid (III) they found that all attempts to hydrolyse (II) to (III) failed. Similarly in the present work, although some impure β-mesaconamic acid was obtained from (II) the yield was only 3% and the method proved to be impracticable.

Cocker and Fateen claimed to have prepared β-methylsuccinamic acid by hydrogenating ethyl β-mesaconamate (II) and hydrolysing the resulting ethyl β-methylsuccinamate with alcoholic potassium hydroxide. When this synthesis was attempted in the present work the only product isolated was α-methylsuccinimide. Similar results have been obtained by Sondheimer and Holley[25] when they attempted to hydrolyse carbobenzoxy-L-aspartamine methyl esters and carbobenzoxy-L-glutamine methyl esters with methanolic sodium hydroxide.

In the present work β-methylsuccinamic acid was prepared from mesaconic β-benzyl ester (IV) by a modification of Anschutz's original synthesis. This ester function was easily removed by hydrogenolysis during the final hydrogenation step. The reaction scheme is given below.



(IV)

(ii) Hydrolysis of β-Cyano-acids

The ease of hydrolysis of the β-cyano-acids in aqueous solution at room temperature has already been mentioned (see p.3). Wideqvist[7], and Foucaud[26] have shown that by using equimolar quantities of nitrile and water it is possible to isolate the intermediate amic-acid in good yield. In the present work difficulties were experienced when the water hydrolysis of the amic-acid was also fast. Only an impure sample of α,α-dimethylsuccinamic acid was obtained by this method and the compound was eventually prepared by the action of ammonia on α,α-dimethylsuccinamic anhydride.

β,β-Dimethylsuccinamic acid (V), α-phenylsuccinamic acid (VI), and β-phenylsuccinamic acid (VIII) were prepared from the corresponding cyano—acids according to the reaction schemes given below.

β,β-Dimethylsuccinamic Acid

$$(CH_3)_2\text{-}C{=}C\text{-}(CO_2Et)_2 \longrightarrow (CH_3)_2\text{-}\underset{\displaystyle CN}{\underset{|}{C}}\text{-}CH_2\text{-}CO_2Et \longrightarrow$$

$$(CH_3)_2\text{-}\underset{\displaystyle CN}{\underset{|}{C}}\text{-}CH_2\text{-}CO_2H \longrightarrow (CH_3)_2\text{-}\underset{\displaystyle CONH_2}{\underset{|}{C}}\text{-}CH_2\text{-}CO_2H \qquad (V)$$

α-Phenylsuccinamic Acid

$$C_6H_5\text{-}\underset{\displaystyle Br}{\underset{|}{CH}}\text{-}CO_2Et + NC\text{-}CH_2\text{-}CO_2Et \longrightarrow C_6H_5\text{-}\underset{\displaystyle NC\text{-}CH\text{-}CO_2Et}{\underset{|}{CH}}\text{-}CO_2Et \longrightarrow$$

$$C_6H_5\text{-}\underset{\displaystyle CH_2\text{-}CN}{\underset{|}{CH}}\text{-}CO_2Et \longrightarrow C_6H_5\text{-}\underset{\displaystyle CH_2\text{-}CN}{\underset{|}{CH}}\text{-}CO_2H \longrightarrow C_6H_5\text{-}\underset{\displaystyle CH_2\text{-}CONH_2}{\underset{|}{CH}}\text{-}CO_2H \qquad (VI)$$

β-Phenylsuccinamic Acid

$$C_6H_5\text{-}CH{=}C\text{-}(CO_2Et)_2 \longrightarrow C_6H_5\text{-}\underset{\displaystyle CN}{\underset{|}{CH}}\text{-}CH_2\text{-}CO_2Et \longrightarrow$$

$$C_6H_5\text{-}\underset{\displaystyle CN}{\underset{|}{CH}}\text{-}CH_2\text{-}CO_2H \longrightarrow C_6H_5\text{-}\underset{\displaystyle CONH_2}{\underset{|}{CH}}\text{-}CH_2\text{-}CO_2H \qquad (VII)$$

(iii) Diazotisation of Asparagine

Holmberg[27] has prepared α-chlorosuccinamic acid and α-bromosuccinamic acid by diazotisation of L-asparagine in the presence of the potassium halide. This synthesis proved to be satisfactory for these compounds.

(iv) The Action of Ammonia on the Cyclic Anhydride

The cleavage of intramolecular anhydrides by ammonia is the least satisfactory method for the synthesis of the amic-acids of asymmetrically substituted succinic acids, as generally mixtures[26] of the two isomeric amic-acids are obtained.

The reaction of mixed anhydrides with amines has been extensively investigated in regard to their application in peptide synthesis.[28] Emery and Gold have found that the following factors influence the course of the cleavage of the anhydride:

(i) Under otherwise identical conditions the carbonyl C atom with the lowest electron density will acylate the nucleophilic amine.

(ii) If one anhydride component is sterically hindered, the other will react preferentially to form the amide.

However, in hydrophilic solvents discrepancies to these rules may occur. Akabori[29] found that the reaction of acetic.chloroacetic anhydride with aniline in benzene gave 85% chloroacetanilide, while in aqueous acetone 72% acetanilide was obtained. A similar dependance on the solvent was demonstrated by Tanenbaum[30] for the aminolysis of phthalylaspartic anhydride. Under anhydrous conditions phthalylasparagine was formed, but in aqueous ethanol the phthalylisoasparagine predominated.

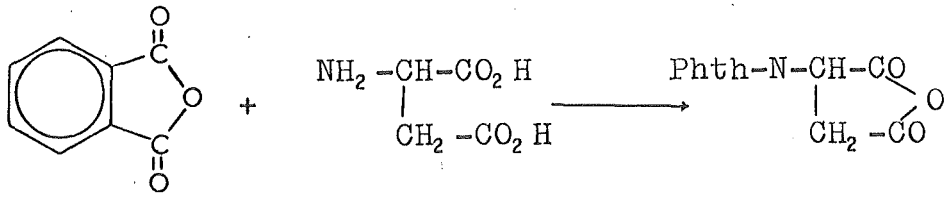
Foucaud's[26] results indicated that the general rules of Emery and Gold were not applicable to the aminolysis of asymmetrically substituted succinic anhydrides. Although the β-amide predominated there appeared to be no definite correlation between the bulk of the substituents and the ratio of the isomers obtained. Foucaud also reported that α,α--dimethylsuccinic anhydride cleaved with ammonia to give 80% of the β-amide and 20% of the α-amide. In the present work the β-amide was prepared from α,α-dimethylsuccinic anhydride as the ammonium salt in 85% yield and shown to be free of the α-isomer by thin layer chromatography.

In the present work the cleavage of methoxy-

succinic anhydride by ammonia showed no dependance on the solvent. In both anhydrous ethereal ammonia, and aqueous ammonia only the β-amide was formed.

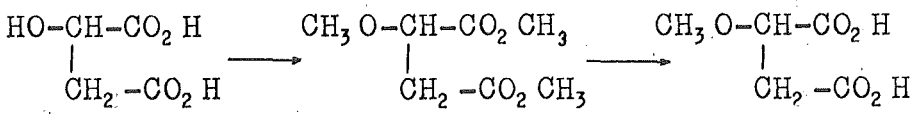
This method was used for the preparation of isoasparagine (VIII), ammonium β-methoxysuccinamate (IX), and ammonium α,α-dimethylsuccinamate (X).

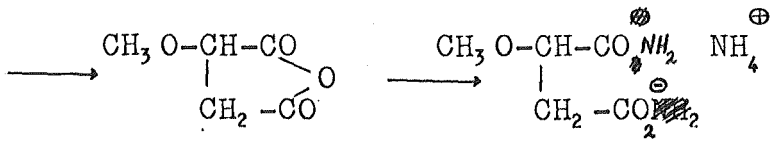
Isoasparagine



(VIII)

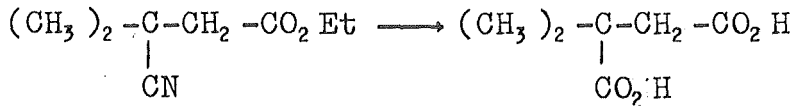
Ammonium β-Methoxysuccinamate

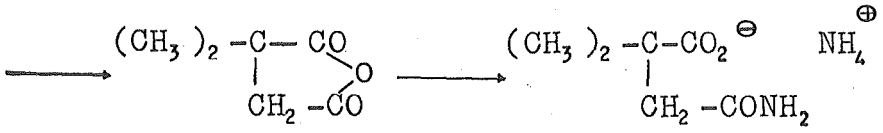




(IX)

Ammonium α,α-Dimethylsuccinamate





(X)

(b) Amic-acids of Symmetrically Substituted Succinic Acids

The most convenient synthesis of the amic-acids in this group is by the action of ammonia on the intramolecular anhydride of the substituted succinic acid. These anhydrides may generally be prepared by the dehydration of the corresponding dibasic acid with

acetyl chloride.

Linstead and Whalley[31] found that it was very difficult to obtain pure meso-α,α'-dimethylsuccinic anhydride. Previous workers[32] had obtained it by the action of acetyl chloride on the meso-acid, but Linstead and Whalley obtained mainly the (±)-isomer by this method. They prepared the meso-anhydride by a milder method involving the action of equimolar quantities of thionyl chloride on the sodium salt of the meso-acid at room temperature.

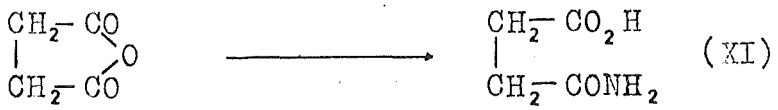
The instability of the meso-anhydride was utilized in this work in the preparation of (±)-α,α'-dimethylsuccinic anhydride. A mixture of meso- and (±)- acids were heated in refluxing acetyl chloride and distilled at atmospheric pressure. Redistillation gave pure (±)-α,α'-dimethylsuccinic anhydride.

The action of ammonia on the cyclic anhydrides of symmetrically substituted succinic acids was used to prepare the following succinamic acids:

succinamic acid (XI),
threo-α,β-dimethylsuccinamic acid (XII),
erythro-α,β-dimethylsuccinamic acid (XIII),

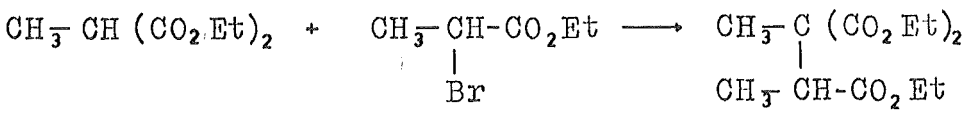
ammonium threo-α,β-dimethoxysuccinamate (XIV), cis-cyclohexane-1,2-dicarboxylic acid mono-amide (XV), trans-cyclohexane-1,2-dicarboxylic acid mono-amide (XVI), cis-cyclopentane-1,2-dicarboxylic acid mono-amide (XVII).

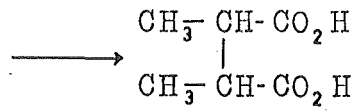
Succinamic Acid



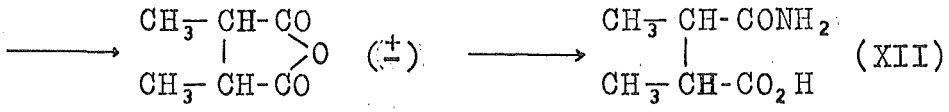
(XI)

threo-α,β-Dimethylsuccinamic Acid



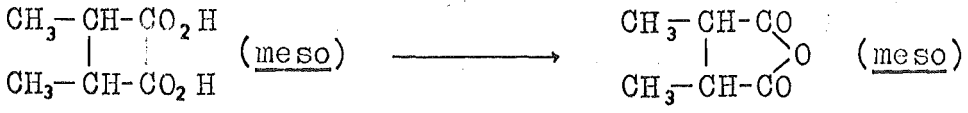


(meso & (±) isomers)

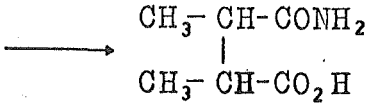


(XII)

erythro-α,β-Dimethylsuccinamic Acid

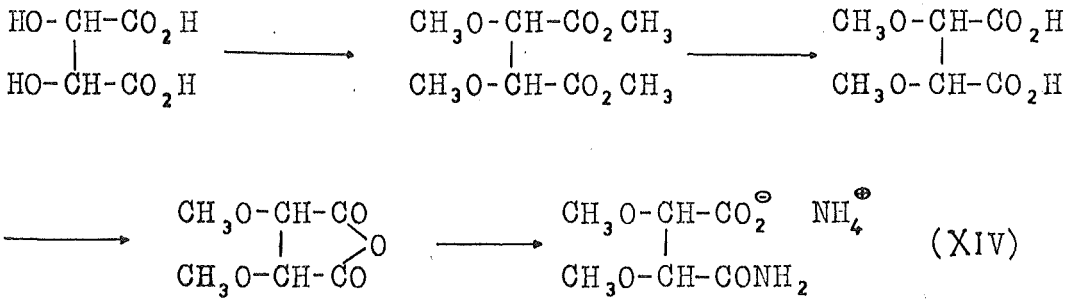


(meso)



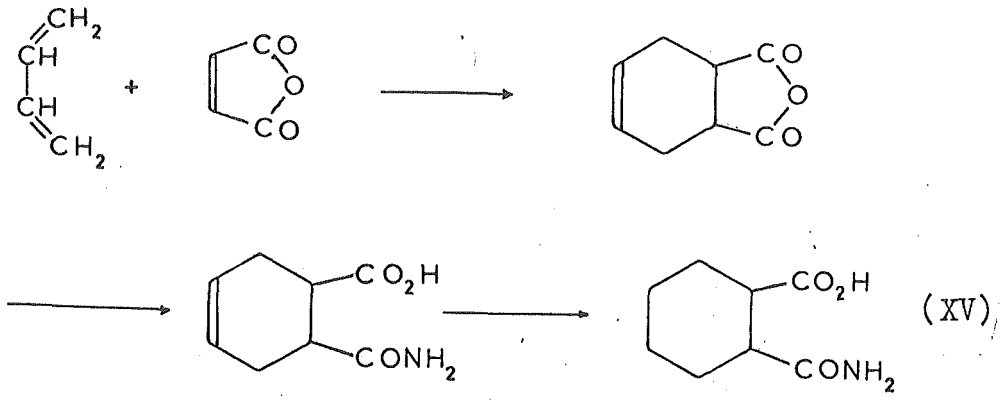
(XIII)

Ammonium threo-α,β-Dimethylsuccimate



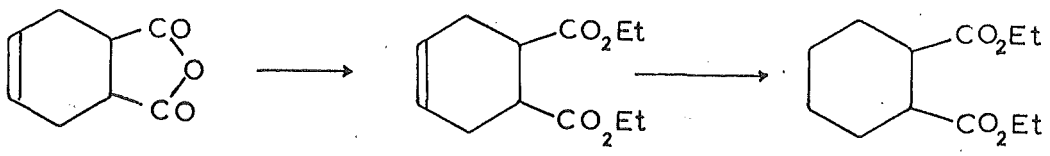
(XIV)

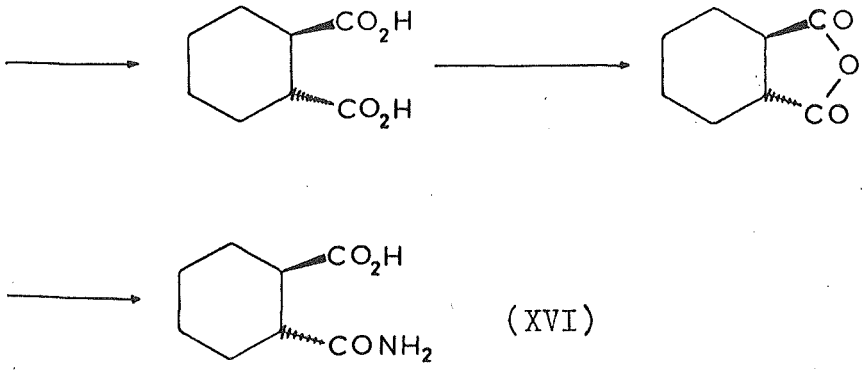
cis-Cyclohexane-1,2-dicarboxylic Acid Mono-amide



(XV)

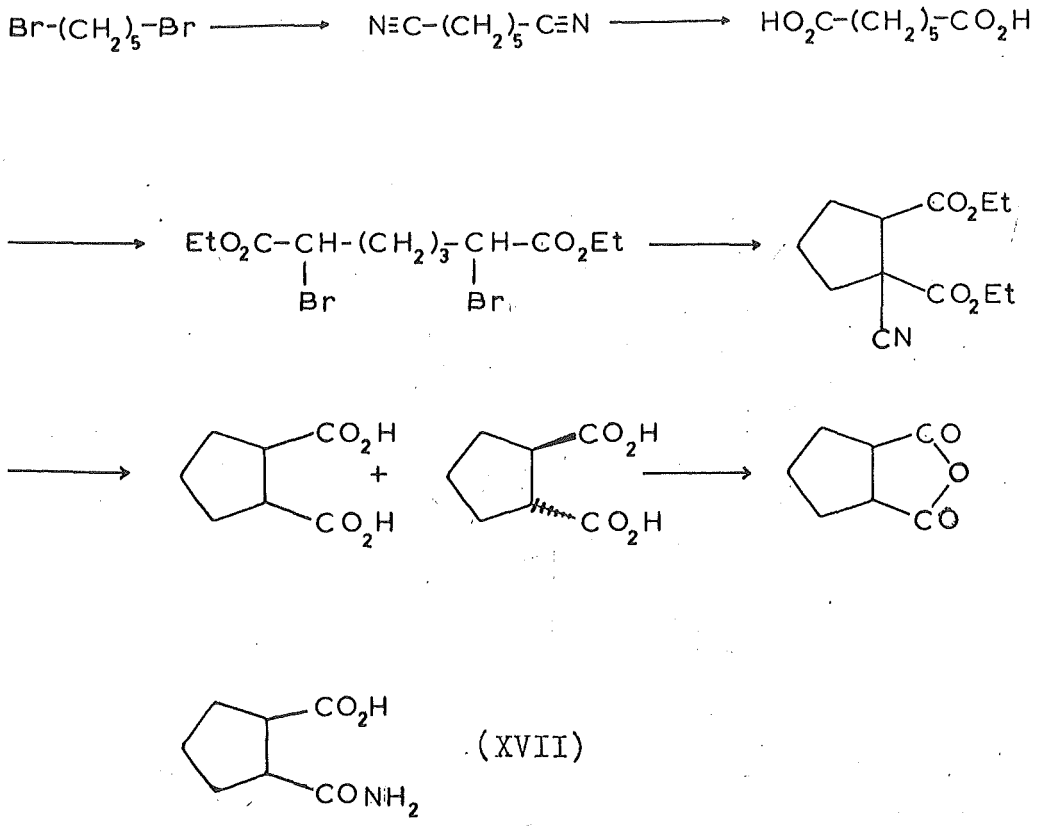
trans-Cyclohexane-1,2-dicarboxylic Acid Mono-amide





(XVI)

cis-Cyclopentane-1,2-dicarboxylic Acid Mono-amide



(XVII)

trans-Cyclopentane-1,2-dicarboxylic acid will not form an anhydride and so it\$ mono-amide was prepared by the ammonolysis of the mono-ethyl ester.

EXPERIMENTAL DETAILS

Preparation of α-Methylsuccinamic Acid

(a) Mesaconic Acid

Mesaconic acid was prepared from citric acid by the method of Shriner, Ford, and Roll.[33] From 800 g. of citric acid 130 g. of mesaconic acid was obtained, m.p. 204-205°, (204°).[33]

(b) Diethylmesaconate

Mesaconic acid (80 g.) was dissolved in ethanol (500 ml.), saturated with anhydrous hydrogen chloride, and left at room temperature for 24 hr. The alcohol was removed on a water bath and the di-ester dissolved in ether. It was then washed successively with saturated sodium bicarbonate solution, and water, dried, and distilled, b.p. 70-72°/0.6 mm. Yield 71 g. (83%).

(c) α-Ethyl hydrogen mesaconate[24]

Diethylmesaconate (60 g.)) was added to a solution of potassium hydroxide (18 g.) in ethanol at 0°. After 90 min. the potassium salt was collected and washed with ether. The salt was dissolved in water, washed once

with ether and acidified with hydrochloric acid. The mono-ester was taken up in ether, dried, and the ether removed on a water bath. The α-ester was purified by five recrystallizations from pentane, m.p. 67-68° (67°),[24] Yield 36 g. (70%).

(d) α-Mesaconamic acid[24]

α-Ethyl hydrogen mesaconate (35 g.) was dissolved in aqueous ammonia (100 ml.d=0.880) and kept at room temperature for 48 hr. The solution was concentrated under reduced pressure and the residue washed with ethanol, dissolved in water, and acidified with hydrochloric acid. The mono-amide was recrystallized from water, m.p. 222-223° (222°),[24] Yield 24.5 g. (83%).

(e) α-Methylsuccinamic acid[34]

α-Mesaconamic acid (20 g.), suspended in ethanol, was hydrogenated at room temperature over platinium oxide (2.g.) at 100 p.s.i. pressure for 1 hr. The amic-acid was crystallized from ethanol, m.p. 127-128° (126-127°).[34] (Found: C, 45.9: H, 6.9: O, 36.2. Calc. for $C_5H_9NO_3$: C, 45.8: H, 6.9: O, 36.6%).

Preparation of β-Methylsuccinamic Acid

(a) Ethyl β-mesaconamate[24]

Phosphorous pentachloride (35 g.) was added to a stirred solution of α-ethyl hydrogen mesaconate (20 g.) in anhydrous ether. When all the phosphorus pentachloride had dissolved the ethereal solution was added dropwise to aqueous ammonia (200 ml. d=0.880). After 15 min. the excess of ammonia was removed under reduced pressure, and the amido-ester extracted with ether. Recrystallization from ether gave plates, m.p. 77-78° (78°),[24] yield 18.9 g. (73%).

(b) Hydrolysis of ethyl β-mesaconamate[23]

A solution of potassium hydroxide (1.71 g.) and ethyl β-mesaconamate (5 g.) in methanol (20 ml.) was kept at room temperature for 12 hr. The solution was neutralized with concentrated sulphuric acid and filtered. The filtrate was evaporated and the residual gum crystallized from water to give 0.13 g. of impure β-mesaconamic acid, m.p. 162-169° (174°).[23]

Thin layer chromatography showed that the mother liquor contained mostly unchanged amido-ester together with smaller amounts of α-ethyl hydrogen mesaconate,

and mesaconic acid. Prolonging the reaction time or increasing the temperature only increased the amounts of α-ethyl hydrogen mesaconate and mesaconic acid without significantly improving the yield of the β-amide.

(c) Ethyl β-methylsuccinamate[24]

Ethyl β-mesaconamate (26 g.) was hydrogenated in ethanol in the presence of palladium on charcoal (5%, 0.5 g.) at 60° and 1 atmosphere pressure. The catalyst was filtered off and the filtrate evaporated to yield 25.9 g. of ethyl β-methylsuccinamate.

(d) Hydrolysis of ethyl β-methylsuccinamate[24]

A solution of potassium hydroxide (7.1 g.) and ethyl β-methylsuccinamate (20 g.) in methanol was refluxed for 2 hr. The solvent was removed and the residue dissolved in water and extracted with ether. The aqueous phase was then acidified with sulphuric acid and the solution continuously extracted with ether for 4 hr. The ethereal solution was dried and evaporated and the α-methylsuccinimide recrystallized from ether, m.p. 68-69° (61-64°)[35], yield 11.1 g. (78%). (Found: C, 53.5: H, 6.4: N, 12.0: O, 28.2. Calc. for $C_5H_7NO_2$: C, 53.1: H, 6.2: N, 12.4: O, 28.3%).

The hydrolysis was repeated at room temperature for 24 hr. and worked up in an identical manner to yield α-methylsuccinimide in 80% yield.

(e) Mesaconyl chloride

Redistilled thionyl chloride (250 g.) and mesaconic acid (100 g.) were refluxed for 3 hr and distilled. Mesaconyl chloride was collected at 64-66°/14mm. Yield 96 g. (75%).

(f) Dibenzyl mesaconate

Mesaconyl chloride (90 g.) was added to a solution of redistilled benzyl alcohol (150 ml.) in anhydrous ether (500 ml.) and left at room temperature for 48 hr. The solution was washed with saturated sodium bicarbonate solution, dried, and distilled, b.p. 150-155°/0.2 mm., yield 130 g. (70%).

(g) Benzyl β-mesaconamate

Dibenzyl mesaconate (65 g.) was added to a suspension of finely powdered potassium hydroxide (11.8 g.) in benzyl alcohol (200 ml.). After 48 hr. at 0° the potassium salt was collected, washed well with ether, dissolved in water, and acidified with hydrochloric acid. The mono-ester was

taken up in ether, and dried. Phosphorus pentachloride (45 g.) was added, and the solution was stirred for 1 hr. before being added slowly to aqueous ammonia (200 ml., d=0.880). The ether and the excess of ammonia was removed under reduced pressure and the amido-ester extracted with ether, dried, and the ether removed in vacuo. Recrystallization from benzene gave 23 g. of benzyl β-mesaconamate, m.p. 116-117°.

(h) β-Methylsuccinamic acid

Benzyl β-mesaconamate (20 g.) in ethanol was hydrogenated over palladium on charcoal (5%, 2 g.) at room temperature and 1 atmosphere. The amic-acid was recrystallized from ethanol, m.p. 134-135° (125°)[24], yield 18.6 g. (90%). (Found: C, 46.1: H, 7.0: O, 36.2. $C_5H_9NO_3$ requires: C, 45.8: H, 6.9: O, 36.6%).

Preparation of β,β-Dimethylsuccinamic Acid

(a) Diethyl isopropylidenemalonate[36]

Ethyl malonate (100 g.), acetone (80 ml.), acetic anhydride (80 ml.), and anhydrous zinc chloride (12 g.) were refluxed gently for 24 hr. The mixture was cooled, diluted with benzene (200 ml.) and washed with water.

The aqueous washings were extracted with benzene, and the combined organic layers dried, and distilled, b.p. 110-120°/15 mm., yield 70 g. (56%).

(b) Ethyl β-cyanoisovalerate[37]

Diethyl isopropylidenemalonate (65 g.), potassium cyanide (16 g.), and 96% ethanol (600 ml.) were refluxed for 10 hr. The solution was cooled and the precipitated potassium bicarbonate filtered off and washed with ethanol. The combined filtrates were made slightly acid with hydrochloric acid and the alcohol removed under reduced pressure. The residue was treated with water and extracted with ether. The extract was then washed with saturated sodium bicarbonate solution, dried, and distilled. Ethyl β-cyanoisovalerate distilled at 60-65°/2 mm., yield 27.6 g. (56%).

(c) β-Cyanoisovaleric acid[37]

Ethyl β-cyanoisovalerate (27 g.) was added to a solution of sodium (4 g.) in 90% aqueous methanol (44 ml.) and kept at room temperature for 5 days. The sodium salt, which was crystallized by the addition of ether, was acidified with dilute sulphuric acid and extracted with ether. The ethereal phase was dried, evaporated

to dryness, and the β-cyanoisovaleric acid crystallized from benzene, m.p. 79-80° (78-79°),[37] yield 18.2 g. (82%).

(d) β,β-Dimethylsuccinamic acid

β-Cyanoisovaleric acid (17 g.) and water (3 ml.) were left at room temperature in a sealed flask for 6 days. The resulting amic-acid was recrystallized from acetone, m.p. 134-135° (134-135°),[26] yield 18.9 g. (98%). (Found: C, 49.7: H, 7.6: O, 33.4. Calc. for $C_6H_{11}NO_3$: C, 49.6: H, 7.6: O, 33.1%)

Preparation of α,α-Dimethylsuccinamic Acid

(a) α-Bromoisobutyric acid

Phosphorus trichloride (5 g.) was added cautiously to a mixture of redistilled isobutyric acid (180 g.) and bromine (288 g.). After 20 hr. at 80° the α-bromoisobutyric acid was distilled, b.p. 115-120°/15 mm., yield 232 g. (81%).

(b) Ethyl α-bromoisobutyrate

α-Bromoisobutyric acid (200 g.) was dissolved in ethanol (200 ml.) and saturated with anhydrous hydrogen chloride. After 24 hr. at room temperature the excess of ethanol was removed and the ester washed with sodium bicarbonate solution. The aqueous washings were

extracted with chloroform and the combined organic layers dried with calcium chloride and distilled. Ethyl α-bromoisobutyrate distilled at 80-85°/15 mm., yield 164 g. (76%).

(c) Diethyl α,α-dimethyl-α'-cyanosuccinate

Ethyl cyanoacetate (120 g.) was added to a refluxing solution of sodium (17 g.) in ethanol (400 ml.). When this addition was completed ethyl α-bromoisobutyrate (150 g.) was added over a period of 90 min. and the refluxing continued for a further 3 hr. The cooled solution was filtered and the sodium bromide washed well with more alcohol. The combined filtrates were then concentrated under reduced pressure and the resulting oil taken up in ether, washed with water, dried, and distilled, b.p. 100-110°/0.5 mm., yield 100 g. (69%)

(d) Ethyl β-cyanopivalate

Diethyl α,α-dimethyl-α'-cyanosuccinate (100 g.) was added to a solution of sodium (10 g.) in 90% aqueous ethanol (90 ml.) and kept at room temperature for 2 days. The sodium salt was collected, washed with ether, and acidified with dilute sulphuric acid. The resulting oil was dissolved in ether, washed with

water, and dried. After the removal of the solvent the mono-ester was decarboxylated in the presence of cupric acetate (0.2 g.) by heating on an oil bath at 140-150° for 5 hr. Ethyl β-cyanopivalate distilled at 80-85°/0.2 mm., yield 43 g. (63%).

(e) β-Cyanopivalic acid

Ethyl β-cyanopivalate was hydrolysed in a similar manner to ethyl β-cyanoisovalerate and crystallized from benzene/pentane, m.p. 69-70°, yield 26 g. (79%).

(f) α,α-Dimethylsuccinamic acid

β-Cyanopivalic acid (20 g.) and water (4 ml.) were left for 7 days at room temperature in a sealed flask. The oil was taken up in ether, dried, and chilled for 4 days in a refrigerator when some crystals of α,α-dimethylsuccinamic acid were obtained, (1.6 g.) m.p. 114-117°. Recrystallization from acetone gave 1.2 g., m.p. 119-120°. (Found: C, 49.0: H, 7.8: O, 32.1. $C_6H_{11}NO_3$ requires: C, 49.6: H, 7.6: O, 33.1%).

The mother liquors were shown to contain mainly the unchanged β-cyanopivalic acid by T.L.C. Prolonged treatment of the β-cyanopivalic acid with water gave rise to the mono-ammonium salt of α,α-dimethylsuccinic acid.

Preparation of α-Phenylsuccinamic Acid

(a) Ethyl phenylbromoacetate

Phenylacetic acid (100 g.), thionyl chloride (75 ml.), and ether (200 ml.) were refluxed on a water bath for 45 min. The solvent was removed, and the acid chloride poured slowly, with stirring, into ethanol (500 ml.). The excess of alcohol was then removed under reduced pressure and the ester dissolved in carbon tetrachloride, washed with sodium bicarbonate solution, and dried with calcium chloride. The ester was then brominated[38] by N-bromosuccinimide (140 g.) in refluxing carbon tetrachloride. After 5 hr. the suspension was filtered, and the filtrate washed with sodium bisulphite solution, dried, and distilled, b.p. 120-125°/2 mm., yield 155 g. (87%).

(b) Diethyl α-phenyl-α'-cyanosuccinate[39]

Ethyl phenylbromoacetate (100 g.) was added slowly to a refluxing solution of sodium ethoxide (29.8 g.) and ethyl cyanoacetate (50 g.) in ethanol (150 ml.). The mixture was refluxed for 3 hr. and worked up in a similar method to that used for diethyl α,α-dimethyl--α'-cyanosuccinate. The product distilled at 180-190°/5 mm., yield 70 g. (62%).

(c) Ethyl α-phenyl-β-cyanopropionate[39]

Diethyl α-phenyl-α'-cyanosuccinate (60 g.) was added to a solution of potassium hydroxide (12.3 g.) in ethanol (70 ml.). After 3 days the solvent was removed and the residue dissolved in water, washed with ether, and acidified. The mono-ethyl α-cyano-α'-phenylsuccinate was extracted with ether, dried, and the ether removed. The acid was then decarboxylated in the presence of cupric acetate (0.2 g.) by heating in vacuo. The product distilled over at 140-160°/5 mm., yield 32 g. (72%).

(d) α-Phenyl-β-cyanopropionic acid[39]

Ethyl α-phenyl-β'-cyanopropionate (30 g.) was added to a solution of sodium (3.8 g.) in 75% aqueous ethanol (80 ml.). After 24 hr. the sodium salt was collected, washed with ether, and dissolved in a minimum amount of water. The solution was acidified with dilute sulphuric acid, extracted with benzene, dried, and concentrated in vacuo. The acid was recrystallized from benzene, m.p. 97-98° (97°[39]), yield 22 g. (85%).

(e) α-Phenylsuccinamic acid

α-Phenyl-β-cyanopropionic acid (20 g.) was dissolved in concentrated sulphuric acid (7 ml.) and the mixture

poured on to ice. The amic-acid was collected, washed well with chilled water, and recrystallized from water, m.p. 159-160° (145°),[40] yield 15.1 g. (68%). (Found: C, 62.1: H, 5.7: O, 25.2. $C_{10}H_{11}NO_3$ requires: C, 62.2: H, 5.7: O, 24.9%).

Preparation of β-Phenylsuccinamic Acid

(a) Ethyl benzalmalonate[41]

A solution of ethyl malonate (100 g.), benzaldehyde (76 g.), and piperidine (5 ml.), in benzene (200 ml.) was refluxed for 16 hr. while the water was removed by a Dean and Stark tube. The solution was washed successively with dilute hydrochloric acid, and sodium bicarbonate solution, dried, and distilled. Ethyl benzalmalonate was collected at 140-144°/4 mm., yield 150 g. (84%).

(b) Ethyl β-phenyl-β-cyanopropionate

Ethyl β-phenyl-β-cyanopropionate was prepared in a similar manner to ethyl-β-cyanoisovalerate. One hundred and thirty grams of ethyl benzalmalonate yielded 76 g. of ethyl β-phenyl-β-cyanopropionate, b.p. 125-130°/0.5 mm.

(c) β-Phenyl-β-cyanopropionic acid

Ethyl β-phenyl-β-cyanopropionate (40 g.) was hydrolysed in a similar manner to ethyl α-phenyl-β-cyanopropionate, m.p. 75-76° (75°)[42], yield 28.8 g. (84%).

(d) β-Phenylsuccinamic acid[42]

β-Phenyl-β-cyanopropionic acid (17.5 g.) and water (1.8 ml.) were kept in a sealed flask for 8 days at room temperature. The resulting solid was washed well with ether and recrystallized from acetone, m.p. 162-164° (158-159°)[42], yield 18 g. (93%). (Found: C, 62.2: H, 5.8: O, 25.1 Calc. for $C_{10}H_{11}NO_3$: C, 62.2: H, 5.7: O, 24.8%).

Preparation of α-Chlorosuccinamic Acid[27]

Sodium nitrite (23 g.) was added slowly over 2 hr to a stirred solution of asparagine (30 g.), and sodium chloride (50 g.) in 2 M hydrochloric acid (200 ml.) at -5°. When the addition was completed 8 g. of concentrated sulphuric acid was added and the solution left for 1 hr to crystallize. The α-chlorosuccinamic acid was collected, washed with chilled water, and recrystallized from water, m.p. 133-134° (130-131°)[27], yield 17.1 g. (84%).

Preparation of α-Bromosuccinamic Acid[43]

α-Bromosuccinamic acid was prepared in a similar manner to α-chlorosuccinamic acid, by the addition of sodium nitrite (20 g.) to a solution of asparagine (30 g.) and potassium bromide (80 g.) in 3 M sulphuric acid (300 ml.). The α-bromosuccinamic acid was recrystallized from water, m.p. 145-146° (146°),[43] yield 20.6 g. (83%).

Preparation of Isoasparagine

(a) Phthalyaspartic anhydride[44]

Aspartic acid (60 g.) and phthalic anhydride (60 g.) were suspended in dry pyridine (120 ml.) and refluxed for 3 hr. with vigorous stirring. The pyridine was removed under reduced pressure on a water bath and the residue treated with acetic anhydride (80 ml.) at 70-80° for 20 min. The solution was cooled and the phthalylaspartic anhydride collected. The mother liquors were diluted with ether to yield another crop of crystals. The anhydride was recrystallized from dioxane/ether, m.p. 212-214° (220-221°),[44] yield 104 g.(88%).

(b) Phthalylisoasparagine[30]

Phthalylaspartic anhydride (30 g.) was dissolved in aqueous ammonia (50 ml., d=0.880). After 5 min.

the solution was concentrated under reduced pressure, and the residue redissolved in a minimum amount of water, and acidified with hydrochloric acid. Phthalylisoasparagine was induced to crystallize by cooling to 0° overnight. Recrystallization from water yielded 24.2 g. of phthalylisoasparagine, m.p. 220-222° (220-222°).[30]

(c) Isoasparagine

Phthalylisoasparagine (20 g.), sodium carbonate (9 g.) and 98% hydrazine hydrate (6 ml.) were dissolved in 75% aqueous ethanol (500 mL) and the solution refluxed for 2 hr. The solution was concentrated to 200 ml. and acidified with acetic acid until no more phthalylhydrazide was precipitated. The suspension was digested on a water bath for 10 min. and then filtered. Isoasparagine was crystallized from the filtrate by the gradual addition of acetone. After two recrystallizations from water/acetone the isoasparagine was shown by thin layer chromatography to be free of asparagine. Yield 3.8 g. (38%).
(Found: N, 20.8. Calc. for $C_4H_8N_2O_3.H_2O$: N, 21.2%)

Preparation of Ammonium β-Methoxysuccinamate

(a) Methyl methoxysuccinate[45]

Malic acid (100 g.) was dissolved in methanol

saturated with hydrogen chloride (500 ml.). After 24 hr. at room temperature the methanol was removed on a water bath and the di-ester distilled, b.p. 125-130°/18 mm., yield 85 g. The methyl malate (85 g.) was dissolved in methyl iodide (250 g.) and silver oxide was added portionwise over 1 hr. When the addition was complete the mixture was refluxed for a further 1.5 hr. and the excess of methyl iodide distilled off. The methyl methoxysuccinate was extracted from the silver residues by ether, using a Soxhlet extractor, and distilled, b.p. 112-116°/14 mm., yield 67.5 g. (73%).

(b) Methoxysuccinic acid[46]

A solution of methyl methoxysuccinate (67 g.) and sodium hydroxide (34 g.) in ethanol (200 ml.) was refluxed for 3 hr. The solution was acidified with hydrochloric acid, evaporated to dryness, and the residue was extracted by ether in a Soxhlet extractor. The acid was recrystallized from ether, m.p. 107-108° (108°),[46] yield 48.5 g. (86%).

(c) Methoxysuccinic anhydride[47]

Methoxysuccinic acid (40 g.) was refluxed with acetyl chloride (200 g.) for 4 hr., and distilled, b.p. 140-150°/20 mm., yield 33.4 g. (95%).

(d) Ammonium β-methylsuccinamate

(i) Methoxysuccinic anhydride (10 g.) was dissolved in anhydrous ether (200 ml.) and saturated with anhydrous ammonia at 0°. The ammonium salt was collected and recrystallized from 96% ethanol, m.p. 164-165°, yield 10.1 g. (80%).

(ii) Methoxysuccinic anhydride (10 g.) was added portionwise to aqueous ammonia (100 ml., d=0.880) and the solution evaporated to dryness under a reduced pressure. The residue was crystallized from 96% ethanol, m.p. 164-165° (mixed m.p. with product from (i) was undepressed) (Found: C, 37.0: H, 7.3: N, 16.7. $C_5H_{12}N_2O_3$ requires: C, 36.5: H, 7.4: N, 17.1%).

Preparation of Ammonium α,α-Dimethylsuccinamate

(a) α,α-Dimethylsuccinic acid[48]

Concentrated hydrochloric acid (300 ml.) and ethyl β-cyanoisovalerate (40 g.) were refluxed for 4 hr. The cooled solution was saturated with ammonium sulphate, and the solution extracted with ether. After removal of the ether the α,α-dimethylsuccinic acid was crystallized

from concentrated hydrochloric acid, m.p. 141-142° (141-142°),[48] yield 31 g. (81%).

(b) Ammonium α,α-dimethylsuccinamate

α,α-Dimethylsuccinic acid (20 g.) and acetyl chloride (80 g.)) were refluxed for 4 hr. and distilled, the α,α-dimethylsuccinic anhydride being collected at 115-120°/18 mm. The anhydride was dissolved in anhydrous ether and saturated with anhydrous ammonia at 0°. The resulting ammonium salt was collected and recrystallized from ethanol/acetone, m.p. 136-137°, yield 19.2 g. (85%). Thin layer chromatography showed the ammonium α,α-dimethylsuccinamate to be free of the β,β-isomer. (Found: C,45.6: H, 7.2: O, 30.3. $C_6H_{14}N_2O_3$ requires: C, 45.2: H, 7.0: O, 30.1%).

Preparation of Succinamic Acid[49]

Succinic anhydride (100 g.) was added with stirring to aqueous ammonia (60 ml, d=0.880). After 10 min. the solution was concentrated under reduced pressure and acidified with hydrochloric acid. The succinamic acid was collected, washed with chilled water, and recrystallized from acetone/ether, m.p. 157-158° (157°)[49] yield 103 g. (88%).

Preparation of threo-α,β-Dimethylsuccinamic Acid

(a) α,α'-Dimethylsuccinic acid

Ethyl methylmalonate (174 g.) and ethyl α-bromopropionate (181 g.) were added to a solution of sodium (23 g.) in ethanol (400 ml.) and the mixture refluxed for 4 hr. The solution was filtered, and the sodium bromide washed with ethanol. The combined filtrate and washings were concentrated under reduced pressure and the residue treated with water and extracted with ether. The ethereal solution was dried and the ether removed. Ethyl butan-2,2,3-tricarboxylate distilled at 100-110°/0.5 mm. The ester was hydrolysed and decarboxylated in 14 hr. with refluxing hydrochloric acid (200 ml.). After the solution had been concentrated to 75 ml. meso-α,α'-dimethylsuccinic acid was obtained, m.p. 182-190°. Three recrystallizations from water yielded 71 g., m.p. 198-200° (198°).[31] The residues were evaporated to dryness to yield 23 g. of a mixture of meso- and (±)-α,α'-dimethylsuccinic acids, m.p. 104-112°.

(b) (±)-α,α'-Dimethylsuccinic anhydride

Acetyl chloride (80 g.) and the mixture of meso- and (±)-α,α'-dimethylsuccinic acids (20 g.) were refluxed for

3 hr, and distilled. The fraction that boiled at 180-240° was collected and redistilled to yield 11.5 g. of (±)-α,α'-dimethylsuccinic anhydride, b.p. 222-224° (223°).[50]

(c) threo-α,β-Dimethylsuccinamic acid

(±)-α,α'-Dimethylsuccinic anhydride (11 g.) was dissolved in anhydrous ether (100 ml.) and saturated with anhydrous ammonia at 0°. The ammonium salt was collected and dissolved in a minimum amount of water, cooled to -10°, and acidified with concentrated sulphuric acid. The amic-acid was recrystallized from ethanol/acetone, m.p. 162-163° (165-167°).[50] (Found: C, 49.5: H, 7.5: O, 33.1. Calc. for $C_6H_{11}NO_3$: C, 49.6: H, 7.6: O,33.1%).

Preparation of erythro-α,β-Dimethylsuccinamic Acid

(a) meso-α,α'-Dimethylsuccinic anhydride[31]

Sodium meso-α,α'-dimethylsuccinate (30 g.) was suspended in anhydrous ether (200 ml.) and thionyl chloride (18.9 g.) was added dropwise. After 12 hr. at 5° the sodium salt was removed by centrifuging and the solvent evaporated at 20° under reduced pressure to yield a crystalline anhydride, (m.p. 39-40°),[30] yield 15.4 g. (76%).

(b) erythro-α,β-Dimethylsuccinamic acid

meso-α,α'-Dimethylsuccinic anhydride (15 g.) was converted to the amic-acid in an identical manner to threo-α,β-dimethylsuccinamic acid. Yield, 12.5 g. (74%), m.p. 147-148° (148-149°).[50] (Found: C, 49.6: H, 7.6: O, 33.2. Calc. for $C_6H_{11}NO_3$: C, 49.6: H, 7.6: O, 33.1%).

Preparation of Ammonium threo-α,β-Dimethoxysuccinamate

(a) (+)-α,α'-Dimethoxysuccinic acid[51]

(+)-Tartaric acid (100 g.) was dissolved in methanol saturated with hydrogen chloride (500 ml.). After 24 hr. at room temperature the methanol was removed and the di-ester distilled, b.p. 112-118°/5 mm. The ester was then dissolved in methyl iodide (400 g.) and the solution refluxed while silver oxide (400 g.) was added portionwise over 1 hr. The heating was continued for a further 30 min. and the excess of methyl iodide removed by distillation. The residual mass was extracted by ether in a Soxhlet extractor, dried, and distilled, to yield 68.8 g. of methyl (+)-α,α'-dimethoxysuccinate, b.p. 127-137°/18 mm.

The ester was hydrolysed by heating in a slight excess of sodium hydroxide for 3 hr. The solution was acidified with hydrochloric acid, and evaporated to

dryness under reduced pressure. The residue was then extracted by ether in a Soxhlet extractor, and the acid crystallized from acetone/hexane, m.p. 152-153° (153°),[51] yield 47 g. (40%).

(b) (+)-α,α'-Dimethoxysuccinic anhydride[47]

(+)-α,α'-Dimethoxysuccinic acid (20 g.) was suspended in acetyl chloride (80 g.) and the mixture refluxed for 3 hr. (+)-α,α'-Dimethoxysuccinic anhydride was distilled under reduced pressure and recrystallized from anhydrous ether/pentane, b.p. 150-155°/14 mm., m.p. 82-84° (80-82°),[47] yield 14.9 g. (83%).

(c) Ammonium (±)-threo-α,β-dimethoxysuccinamate

(+)-α,α'-Dimethoxysuccinic anhydride (12 g.) was dissolved in anhydrous ether and treated with anhydrous ammonia. The ammonium salt was recrystallized from anhydrous ethanol/ether to yield hygroscopic needles, m.p. 176-178°, yield 8 g. (55%). Recrystallization from aqueous ethanol gave the stable mono-hydrate of the ammonium salt, m.p. 122-123°. (Found: C, 34.2: H, 7.6: O, 44.9: $C_6H_{14}N_2O_5.H_2O$ requires: C, 34.0: H, 7.6: O, 45.2%).

Preparation of cis-Cyclohexane-1,2-dicarboxylic Acid Mono-amide

(a) cis-Cyclohex-4-ene-1,2-dicarboxylic anhydride

1,3-Butadiene, prepared by the method of Hershberg and Ruhoff[52] by the catalytic cracking of cyclohexene (200 ml.) over a chromel A heating ribbon at a bright red heat, was purified by bulb to bulb distillation. Yield 78 g. (73%). The butadiene was then passed into a solution of maleic anhydride (98 g.) in dry benzene (200 ml.) at 60-70°. After 3 hr. the uptake of butadiene had ceased and the solution was allowed to crystallize at 0-5°, m.p. 101-102° (103-104°),[53] yield 140 g. (92%).

(b) cis-Cyclohex-4-ene-1,2-dicarboxylic acid mono-amide

cis-Cyclohex-4-ene-1,2-dicarboxylic anhydride (50 g.) was added to a solution of aqueous ammonia (200 ml., d=0.880) at 0°, stirred for 15 min., and concentrated to 100 ml. at 60° under reduced pressure. The solution was acidified with concentrated sulphuric acid at -10° and the resulting amic-acid collected, washed well with chilled water, and recrystallized from ethanol, m.p. 152-153°, yield 37 g. (67%).

(c) cis-Cyclohexane-1,2-dicarboxylic acid mono-amide

cis-Cyclohex-4-ene-1,2-dicarboxylic acid mono-amide (20 g.) was hydrogenated in ethanol over palladium on charcoal (5%, 2 g.) at 60° and 1 atmosphere pressure. The catalyst was filtered off and washed 3 times with hot ethanol. The combined filtrate and washings were then concentrated to 200 ml. and left to crystallize. The amic-acid was recrystallized from ethanol, m.p. 177-178 (178°),[54] yield 17.8 g. (88%).

Preparation of trans-Cyclohexane-1,2-dicarboxylic Acid Mono-Amide

(a) Ethyl cis-cyclohexane-1,2-dicarboxylate

Ethyl cis-cyclohex-4-ene-1,2-dicarboxylate (120 g.), prepared by the method of Cope and Herrick[55] from cis--cyclohex-4-ene-1,2-dicarboxylic anhydride (100 g.), was hydrogenated in ethanol over palladium on charcoal (5%, 2 g.) at 60° and 1 atmosphere pressure. The catalyst was removed and the di-ester distilled, b.p. 156-158°/18 mm., yield 115 g. (95%).

(b) trans-Cyclohexane-1,2-dicarboxylic acid[56]

A solution of ethyl cis-cyclohexane-1,2-dicarboxylate (100 g.) and potassium hydroxide (60 g.) in ethanol (600 ml.)

was refluxed for 4 hr. Most of the alcohol was removed and replaced with water, and the heating continued for a further 3 hr. The cooled solution was acidified with hydrochloric acid and the resulting acid recrystallized from water, m.p. 219-220° (219-220°),[56] yield 54 g. (85%).

(c) trans-Cyclohexane-1,2-dicarboxylic anhydride

trans-Cyclohexane-1,2-dicarboxylic acid (50 g.) and acetyl chloride (100 g.) were refluxed for 3 hr. and the excess of acetyl chloride and acetic anhydride was removed under reduced pressure. The residual anhydride was crystallized from anhydrous ether, m.p. 143-145° (143-144°),[57] yield 37.2 g. (83%).

(d) trans-Cyclohexane-1,2-dicarboxylic acid mono-amide

trans-Cyclohexane-1,2-dicarboxylic anhydride (30 g.) was treated with aqueous ammonia (150 ml., d=0.880) and worked up in the usual manner to yield 24 g. of the mono--amide, m.p. 195-196° (196°).[54]

Preparation of cis-Cyclopentane-1,2-dicarboxylic Acid Mono-amide

(a) Pimelic acid[58]

1,5-Dibromopentane (230 g.), potassium cyanide (140 g.),

water (350 ml.), and ethanol (1000 ml.) were refluxed for 4 hr. The solvent was removed and the residue was extracted with benzene, washed successively with sodium hydroxide solution, and water, dried, and distilled. The pimelonitrile distilled at 120-125°/2 mm. The nitrile was hydrolysed in boiling hydrochloric acid (350 ml.) in 2 hr. to yield 150 g. of pimelic acid, m.p. 102-103° (103°).[58]

(b) Ethyl α,α'-dibromopinelate

Pimelic acid (150 g.) and thionyl chloride (250 g.) were refluxed for 4 hr. and the excess of thionyl chloride removed by distillation. The acid chloride was heated to 80° and bromine (300 g.) was added, with stirring, over a period of 8 hr. The mixture was heated for a further 4 hr. and then poured cautiously into ethanol (300 ml.) at 0°. The excess of ethanol was removed and ethyl α,α'-dibromopimelate distilled at 170-175°/2 mm., yield 240 g. (69%).

(c) Ethyl 1-cyanocyclopentane-1,2-dicarboxylate[59]

A solution of ethyl α,α'-dibromopimelate (240 g.) and sodium cyanide (120 g.) in ethanol (200 ml.) were refluxed for 60 hr. and filtered. The residue was washed with more alcohol and the combined filtrate and washings were distilled. Ethyl 1-cyanocyclopentane-

-1,2-dicarboxylate was collected at 125-135°/2 mm., yield 121 g. (79%).

(d) cis-Cyclopentane-1,2-dicarboxylic anhydride[59]

Ethyl 1-cyanocyclopentane-1,2-dicarboxylate (121 g.) and hydrochloric acid (700 ml.) were refluxed for 3 days. The solution was concentrated to 300 ml. and chilled to induce the crystallization of trans-cyclopentane-1,2-dicarboxylic acid, m.p. 162-163° (162-163°),[59] yield 40 g. The mother liquor was evaporated to dryness under reduced pressure and the residue extracted with acetone. After the acetone had been removed the mixture of cis- and trans-acids were refluxed in acetic anhydride (200 ml.) for 10 hr. and distilled. cis-Cyclopentane-1,2-dicarboxylic anhydride was collected at 95-100°/4 mm., yield 21.7 g.

(e) cis-Cyclopentane-1,2-dicarboxylic acid mono-amide

Cyclopentane-1,2-dicarboxylic anhydride (20 g.) was dissolved in anhydrous ether (100 ml.) and saturated with anhydrous ammonia. The amic-acid was obtained from the ammonium salt in the usual manner and recrystallized from ethanol/ether, m.p. 126-128° (126-129°),[60] yield 6.1 g. (27%).

Preparation of trans-Cyclopentane-1,2-dicarboxylic Acid Mono-amide.

(a) Ethyl hydrogen trans-cyclopentane-1,2-dicarboxylate

A solution of trans-cyclopentane-1,2-dicarboxylic acid (40 g.) in toluene (100 ml.) and ethanol (300 ml.) was saturated with anhydrous hydrogen chloride, and refluxed for 4 hr. The solvent was removed and the residue heated again with toluene (100 ml.) and ethanol (200 ml.) saturated with hydrogen chloride, for 1 hr. The solvent was removed and the di-ester distilled at 128-133°/18 mm. The di-ester (47 g.) and sodium hydroxide (8.8 g.) were dissolved in ethanol (100 ml.) and kept at room temperature for 3 days. After the alcohol had been removed the residue was dissolved in water, extracted with ether, and the aqueous phase acidified. The resulting oil was extracted into ether, dried, and distilled. The mono-ester distilled at 135-140°/18 mm., yield 27.8 g. (68%).

(b) trans-Cyclopentane-1,2-dicarboxylic acid mono-amide

Ethyl hydrogen trans-cyclopentane-1,2-dicarboxylate (20 g.) and aqueous ammonia (40 ml., d=0.880) were heated in a sealed tube at 140° for 14 hr. The amic-acid was liberated from the resulting ammonium salt in the usual

manner and recrystallized from ethanol, m.p. 184-185°, yield 13.2 g. (78%). (Found: C, 53.4: H, 7.1: O, 30.4. $C_7H_{11}NO_3$ requires: C, 53.5: H, 7.1: O, 30.6%).

HYDROLYSIS OF SUCCINAMIC ACIDS

General

Standard solutions of hydrochloric acid, sulphuric acid, and sodium hydroxide were obtained by the dilution of Riedel-de Häen "Fixanal" with distilled water.

For all kinetic runs the ionic strength was adjusted to I = 1.0 by the addition of A.R. potassium chloride.

The constant temperature bath, which had a capacity of 17 litres, was filled with Carbowax 400. The temperature was regulated by a Temcan Tempunit to $\pm 0.05°$.

Introduction

Previous workers[10] have shown that when compounds related to succinamic acid hydrolyse as the undissociated acid, they do so according to the expression,

$$k_{obs} = k_I / (1 + K_a/[H^+]) + k_{II}[H^+] \quad \ldots\ldots (1)$$

where K_a is the dissociation constant of the carboxylic acid group.

The unimolecular rate constant, k_I, may be obtained from this system in two ways.

(a) Low acid concentrations

If $k_I \geqslant k_{II}$, then for low acid concentrations the term $k_{II}[H^+]$ may be disregarded and

$$k_{obs} = k_I /(1 + K_a/[H^+])$$

i.e.

$$1/k_{obs} = 1/k_I + K_a/(k_I [H^+])$$

Therefore k_I may be obtained from a plot of $1/k_{obs}$ vs $1/[H^+]$.

If $k_I < k_{II}$ then the contributions from the term $k_{II}[H^+]$ become significant and a curved plot is obtained.

(b) High acid concentrations

At high acid concentrations the term $k_I /(1 + K_a/[H^+])$ tends to k_I and the original expression, (1), simplifies to

$$k_{obs} = k_I + k_{II} [H^+]$$

From this k_I and k_{II} may be obtained simply by plotting k_{obs} vs $[H^+]$.

Methods of analysis

Two methods of analysis have been employed

(a) Direct titration of ammonia

At acid concentrations lower than 3×10^{-2} M the pH of the solution was found to increase during the course of the reaction due to the formation of ammonia. The rate of formation of ammonia could, therefore, be followed by direct titration with relatively concentrated hydrochloric acid.

The pH of 100 ml. of 0.01 M amic-acid solution was adjusted with potassium hydroxide to between 6.5 and 7.5, and the solution placed in the reaction cell in the constant temperature bath. After the solution had come to thermal equilibrium the electrodes were inserted and the solution adjusted to the required pH with preheated 1.0 M hydrochloric acid. The rate of formation of ammonia was then followed using a Radiometer model T.T.T.1c. Titrator to maintain a constant pH by the addition of 0.10 M hydrochloric acid.

A plot of titre vs time gave an exponential curve which was analysed by the method of Guggenheim.[61]

$$k_{obs}t + \ln(V_{(t + \Delta t)} - V_t) = \text{const.}$$

V_t is the titre at time t, and $(V_{(t + \Delta t)})$ is the titre after a time $(t + \Delta t)$. Δt was chosen to be greater than 3 times the half life of the reaction.

The unimolecular rate constant, k_1, was obtained from a graph of $1/k_{obs}$ vs $1/[H^+]$.

The main source of errors in this method is the determination of the pH. The pH meter was calibrated each day against the pH of 0.05 M potassium hydrogen phthalate at the appropriate temperature. The reading accuracy of the meter is of the order of $\pm$0.02 pH units. This gives an overall accuracy for the experimental data of $\pm$0.04 pH units. This error has little effect on k_1, however, as k_{obs} approaches k_I assymtopically with decreasing pH. The errors in K_a are significant and the values determined experimentally during the hydrolysis can only be regarded as approximate.

The reproducibility of this method was checked with α-methylsuccinamic acid at 78.9° and a pH of 2.95, and shown to be better than $\pm$1% ($10^5 k_{obs}$ = 6.38, 6.43, 6.47. sec^{-1}).

(b) Distillation of ammonia

This method was used for acid concentrations which exceeded 3×10^{-2} M. No change in pH was observed during the course of the reaction.

In this method the solutions of amic-acid and hydrochloric acid were mixed at room temperature, and

10 ml. aliquots were pipetted into sample tubes whose stoppers were secured by steel springs. These aliquots were withdrawn from the constant temperature bath, the reaction quenched by cooling in ice, and the contents transferred to the distillation apparatus. The sample tubes were rinsed twice with distilled water. Ten ml. of sodium hydroxide, and 20 ml. of saturated potassium carbonate were added and the ammonia steam distilled at 50°/70 mm. for 15 min. into 20 ml. of 0.005 M sulphuric acid. The ammonia was then determined by back titration of the sulphuric acid with sodium hydroxide using a Radiometer Model T.T.T.1.c Dead Stop Titrator.

Six test runs were conducted using 0.010 M ammonium chloride in 0.60 M hydrochloric acid, and the method was found to be reproducible to $\pm$ 1%.

The errors due to the hydrolysis of the amide in the alkaline solution were checked in each case and found to be negligible.

The reproducibility of a kinetic run analysed by this method was also checked and found to be better than $\pm$ 2%.

For a first order reaction we have,

$$k_{obs} = (2.303/t)\log(a/(a - x)) \quad \ldots\ldots\ldots\ldots (2)$$

where "a" is the initial concentration of the substrate, and "x" is the amount that has reacted in time "t".

If V_o is the initial titre of sodium hydroxide against sulphuric acid, V_∞ the final titre corresponding to the completion of the reaction, and V_t the titre after time "t", then $V_o \alpha c$, $V_\infty \alpha (c - a)$, and $V_t \alpha (c - x)$, and the expression (2) can be rearranged to give,

$$k_{obs} = (2.303/t)\log((V_o - V_\infty)/(V_t - V_\infty))$$

k_{obs} can then be determined from the slope of a plot of $\log (V_t - V_\infty)$ vs t.

Both the unimolecular and bimolecular rate constants, k_I and k_{II}, are determined from the experimental rate constant, k_{obs}, obtained by this method.

The hydrolysis of isoasparagine was measured using both methods of analysis and the unimolecular rate constants, k_I, were found to be in good agreement (see p. 75).

Typical Run

Detailed calculations for a typical run for both methods of analysis are given below.

(a) Direct titration of ammonia

59.7° ~~57°~~ β,β-Dimethylsuccinamic acid was hydrolysed at at a pH of 3.82.

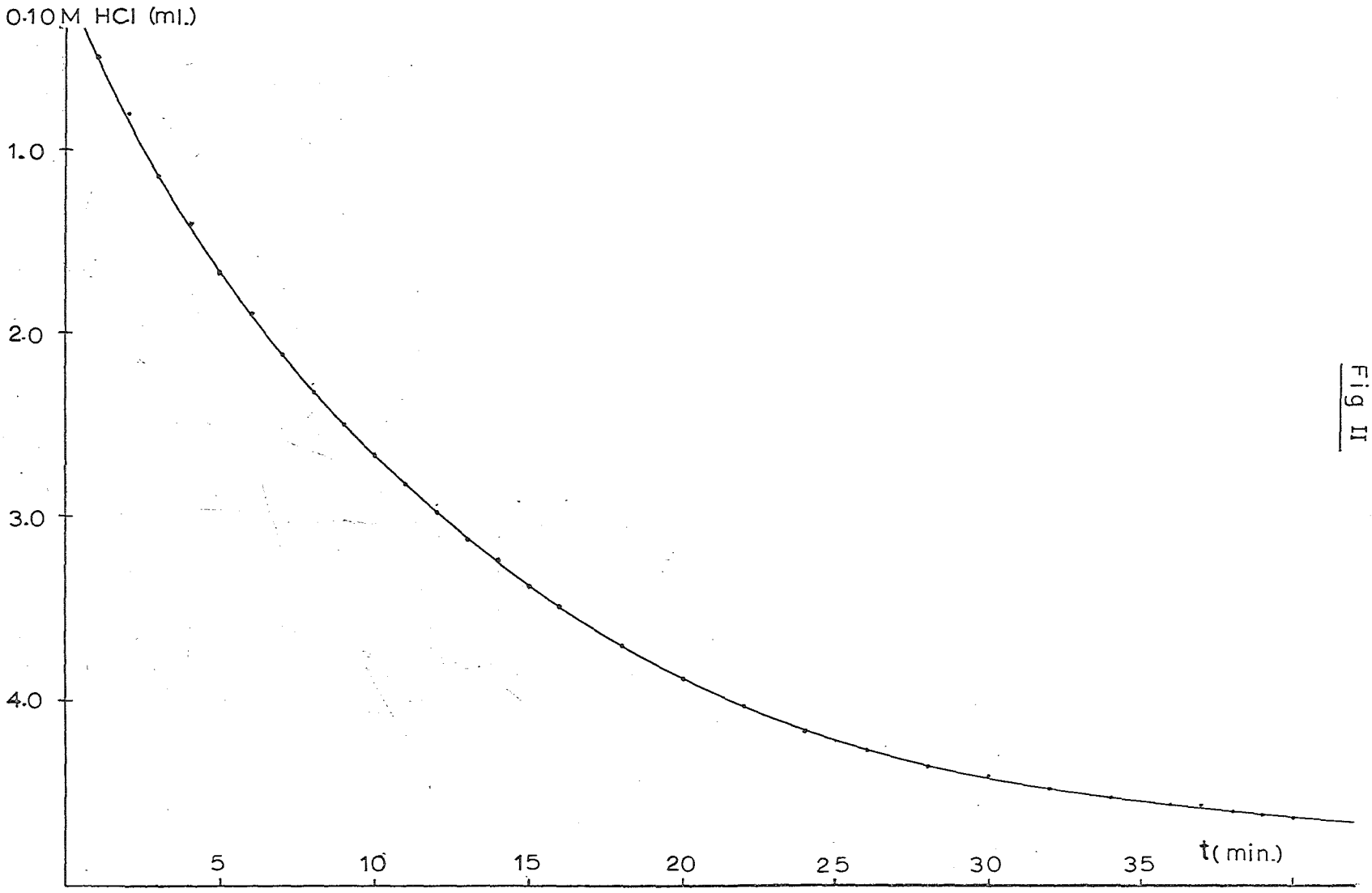


Fig II

The rate of addition of 0.10 M hydrochloric acid is shown in Fig. II. Values of V_t and $V_{(t + \Delta t)}$ were obtained from this curve and the calculations of the rate constant are shown in the Table II below.

Table II

t (min)	V_t	$V_{(t + 24)}$	$V_{(t + 24)} - V_t$	$\log(V_{(t +24)} - V_t)$
1	0.47	4.20	3.73	0.573
2	0.80	4.24	3.44	0.537
3	1.10	4.28	3.18	0.502
4	1.36	4.32	2.96	0.471
5	1.62	4.36	2.74	0.438
6	1.86	4.39	2.53	0.403
7	2.08	4.42	2.34	0.369
8	2.29	4.45	2.16	0.335
9	2.46	4.48	2.02	0.305
10	2.64	4.51	1.87	0.272
11	2.80	4.54	1.74	0.241
12	2.95	4.56	1.61	0.207

A graph of log $(V_{(t+24)} - V_t)$ vs t gave a straight line with slope = 5.50×10^{-4} sec^{-1}. This corresponds to a first-order rate constant of $k_{obs} = 1.25 \times 10^{-4} sec^{-1}$.

(b) Distillation of ammonia

trans-Cyclopentane-1,2-dicarboxylic acid mono-amide was hydrolysed at 85.0° by 0.10 M hydrochloric acid.

The infinity point, V_∞, corresponding to the end of the reaction was determined after 36 hr. in triplicate.

V_∞ = 12.30, 12.31, 12.30.

Table III

t (min)	V_t	$V_t - V_\infty$	$\log(V_t - V_\infty)$
0	20.35	8.05	0.905
8	19.76	7.46	0.873
26	18.74	6.44	0.809
46	18.15	5.85	0.767
79	16.70	4.40	0.644
100	16.15	3.85	0.586
120	15.62	3.31	0.520
144	15.04	2.74	0.438
165	14.63	2.33	0.367
187	14.26	1.96	0.292

A plot of $\log(V_t - V_\infty)$ vs t gives a straight line with a slope = 5.43×10^{-5} sec^{-1}. This corresponds to a first order rate constant $k_{obs} = 1.25 \times 10^{-4}$ sec^{-1}.

RESULTS

The following list of results are for the reactions analysed by the direct titration of ammonia.

(a) cis-Cyclopentane-1,2-dicarboxylic acid mono-amide

30.5°

$10^4 k_{obs}$ (sec^{-1})	$a_{H_3O^+}$ (moles/l.)
2.24	3.090×10^{-4}
2.38	5.370×10^{-4}
2.50	8.128×10^{-4}
2.52	1.445×10^{-3}
2.62	2.572×10^{-3}

$k_I = 2.666 \times 10^{-4}\ sec^{-1}$

$K_a = 1.7 \times 10^{-5}$

41.2°

$10^4 k_{obs}$ (sec^{-1})	$a_{H_3O^+}$ (moles/l.)
7.17	5.25×10^{-5}
8.70	1.259×10^{-4}
8.90	2.754×10^{-4}
9.12	6.310×10^{-4}
9.28	1.950×10^{-3}

$k_I = 9.404 \times 10^{-4}\ sec^{-1}$

$K_a = 6.1 \times 10^{-5}$

51.3°

$10^3 k_{obs}$ (sec^{-1})	$a_{H_3O^+}$ (moles/l.)
1.895	4.90×10^{-5}
2.08	1.000×10^{-4}
2.46	3.020×10^{-4}
2.51	3.420×10^{-4}
2.72	9.550×10^{-4}

$k_I = 2.777 \times 10^{-3}\ sec^{-1}$

$K_a = 3.0 \times 10^{-5}$

(b) cis-Cyclohexane-1,2-dicarboxylic acid mono-amide

51.3°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
5.27	7.94×10^{-5}
5.96	3.802×10^{-4}
6.16	7.431×10^{-4}
6.24	1.230×10^{-3}
6.28	2.344×10^{-3}

$k_I = 6.323 \times 10^{-4}\ sec^{-1}$

$K_a = 1.8 \times 10^{-5}$

60.2°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.962	3.09×10^{-5}
1.467	1.259×10^{-4}
1.515	2.089×10^{-4}
1.530	4.677×10^{-4}
1.600	1.259×10^{-3}
1.660	1.660×10^{-3}

$k_I = 1.661 \times 10^{-3}\ sec^{-1}$

$K_a = 3.5 \times 10^{-5}$

70.0°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
2.99	3.47×10^{-5}
4.02	1.622×10^{-4}
4.07	3.162×10^{-4}
4.10	6.310×10^{-4}
4.25	1.047×10^{-3}
4.37	2.138×10^{-3}

$k_I = 4.35 \times 10^{-3}\ sec^{-1}$

$K_a = 2.7 \times 10^{-5}$

(c) β,β-Dimethylsuccinamic acid

51.6°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
4.21	5.15×10^{-5}
5.70	1.858×10^{-4}
5.83	1.005×10^{-3}
6.00	1.503×10^{-3}

$k_I = 6.154 \times 10^{-4}\ sec^{-1}$

$K_a = 3.3 \times 10^{-5}$

59.7°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.606	3.01×10^{-5}
1.165	4.58×10^{-5}
1.268	1.496×10^{-4}
1.355	8.222×10^{-4}
1.390	1.390×10^{-3}
1.430	2.698×10^{-3}

$k_I = 1.439 \times 10^{-3}\ sec^{-1}$

$K_a = 3.2 \times 10^{-5}$

69.2°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
2.02	4.41×10^{-5}
3.05	2.342×10^{-4}
3.16	1.919×10^{-4}
3.27	2.343×10^{-4}
3.52	1.585×10^{-3}
3.55	2.830×10^{-3}

$k_I = 3.574 \times 10^{-3}\ sec^{-1}$

$K_a = 2.5 \times 10^{-5}$

78.9°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.67	1.48×10^{-5}
5.99	5.89×10^{-5}
7.43	1.479×10^{-4}
7.90	3.090×10^{-4}
8.04	3.284×10^{-4}
8.73	1.318×10^{-3}

$k_I = 8.770 \times 10^{-3}\ sec^{-1}$

$K_a = 2.4 \times 10^{-5}$

(d) α,α-Dimethylsuccinamic acid

51.3°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
4.00	6.03×10^{-5}
4.83	1.660×10^{-4}
5.48	4.365×10^{-4}
5.54	1.259×10^{-3}
5.58	3.311×10^{-3}

$k_I = 5.661 \times 10^{-4}\ sec^{-1}$

$K_a = 2.9 \times 10^{-5}$

60.2°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.916	4.26×10^{-5}
1.280	1.549×10^{-4}
1.335	3.236×10^{-4}
1.401	6.457×10^{-4}
1.430	1.288×10^{-3}
1.441	1.738×10^{-3}

$k_I = 1.457 \times 10^{-3}\ sec^{-1}$

$K_a = 2.5 \times 10^{-5}$

70.0°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.89	3.16×10^{-5}
2.80	9.55×10^{-5}
3.39	3.162×10^{-4}
3.44	3.467×10^{-4}
3.60	9.772×10^{-4}
3.62	1.995×10^{-3}
3.66	2.512×10^{-3}

$k_I = 3.697 \times 10^{-3}\ sec^{-1}$

$K_a = 3.4 \times 10^{-5}$

(e) threo-α,β-Dimethylsuccinamic acid

50.8°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.23	9.55×10^{-5}
3.34	1.047×10^{-4}
4.15	2.399×10^{-4}
4.49	7.079×10^{-4}
4.68	1.288×10^{-3}
4.82	1.778×10^{-3}

$k_I = 4.968 \times 10^{-4}\ sec^{-1}$

$K_a = 6.0 \times 10^{-5}$

60.2°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.667	4.90×10^{-5}
0.972	1.905×10^{-4}
1.15	5.012×10^{-4}
1.18	6.310×10^{-4}
1.20	1.148×10^{-3}
1.215	1.778×10^{-3}

$k_I = 1.246 \times 10^{-3}\ sec^{-1}$

$K_a = 4.4 \times 10^{-5}$

70.0°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.982	3.14×10^{-5}
2.07	1.349×10^{-4}
2.54	3.020×10^{-4}
2.85	7.586×10^{-4}
2.91	8.318×10^{-4}
2.92	1.660×10^{-3}
3.11	3.162×10^{-3}

$k_I = 3.124 \times 10^{-3}\ sec^{-1}$

$K_a = 6.8 \times 10^{-5}$

(f) erythro-α,β-Dimethylsuccinamic acid

60.2°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.34	8.51×10^{-5}
2.50	2.818×10^{-4}
3.01	5.012×10^{-4}
3.33	8.128×10^{-4}
3.60	1.479×10^{-3}

$k_I = 4.025 \times 10^{-4}\ sec^{-1}$

$K_a = 1.7 \times 10^{-4}$

70.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.16	3.72×10^{-5}
5.00	6.61×10^{-5}
6.96	1.660×10^{-4}
8.52	3.162×10^{-4}
9.30	5.248×10^{-4}
9.83	1.380×10^{-3}
9.99	3.715×10^{-3}

$k_I = 1.045 \times 10^{-3}\ sec^{-1}$

$K_a = 7.8 \times 10^{-5}$

78.9°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.58	1.012×10^{-4}
1.93	1.995×10^{-4}
2.16	4.169×10^{-4}
2.22	6.026×10^{-4}
2.34	1.202×10^{-3}
2.41	1.995×10^{-3}

$k_I = 2.458 \times 10^{-3}\ sec^{-1}$

$K_a = 5.4 \times 10^{-5}$

(g) β-Methylsuccinamic acid

68.4°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.41	3.27×10^{-5}
2.34	1.047×10^{-4}
2.48	1.381×10^{-4}
2.56	1.905×10^{-4}
2.70	7.079×10^{-4}
2.78	6.308×10^{-4}
2.89	8.318×10^{-4}

$k_I = 2.992 \times 10^{-4}\ sec^{-1}$

$K_a = 4.4 \times 10^{-5}$

78.4°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
2.90	2.19×10^{-5}
5.30	7.59×10^{-5}
6.60	1.550×10^{-4}
7.04	3.715×10^{-4}
7.45	6.607×10^{-4}

$k_I = 8.417 \times 10^{-4}\ sec^{-1}$

$K_a = 6.0 \times 10^{-5}$

92.0°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.57	7.08×10^{-6}
1.32	5.03×10^{-5}
1.98	1.000×10^{-4}
2.05	1.291×10^{-4}
2.58	2.949×10^{-4}
2.76	3.662×10^{-4}

$k_I = 2.981 \times 10^{-3}\ sec^{-1}$

$K_a = 4.1 \times 10^{-5}$

(h) α-Methylsuccinamic acid

64.7°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.644	5.00×10^{-5}
1.110	1.291×10^{-4}
1.408	6.138×10^{-4}
1.470	8.610×10^{-4}
1.543	2.587×10^{-3}

$k_I = 1.581 \times 10^{-4}\ sec^{-1}$

$K_a = 6.6 \times 10^{-5}$

78.9°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.31	6.07×10^{-5}
4.14	9.95×10^{-5}
4.73	1.135×10^{-4}
5.50	3.470×10^{-4}
5.54	2.260×10^{-4}
5.92	5.131×10^{-4}
6.43	1.135×10^{-3}

$k_I = 6.621 \times 10^{-4}\ sec^{-1}$

$K_a = 5.3 \times 10^{-5}$

92.0°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.634	1.90×10^{-5}
1.81	1.778×10^{-4}
1.99	3.388×10^{-4}
2.16	1.291×10^{-3}
2.27	1.590×10^{-3}
2.40	1.000×10^{-2}
2.44	9.779×10^{-3}

$k_I = 2.365 \times 10^{-3}\ sec^{-1}$

$K_a = 6.3 \times 10^{-5}$

(i) β-Phenylsuccinamic acid

71.6°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.26	6.61×10^{-5}
1.76	3.981×10^{-4}
1.94	7.762×10^{-4}
1.97	8.095×10^{-4}
1.99	1.175×10^{-3}
2.04	1.468×10^{-3}
2.07	3.162×10^{-3}

$k_I = 2.126 \times 10^{-4}\ sec^{-1}$

$K_a = 5.9 \times 10^{-5}$

78.9°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
2.54	6.92×10^{-5}
2.96	2.138×10^{-4}
3.60	2.512×10^{-4}
3.78	4.898×10^{-4}
4.08	5.754×10^{-4}
4.22	1.905×10^{-3}
4.30	2.163×10^{-3}

$k_I = 4.39 \times 10^{-4}\ sec^{-1}$

$K_a = 5.2 \times 10^{-5}$

94.0°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.733	3.39×10^{-5}
1.175	8.32×10^{-5}
1.59	5.316×10^{-4}
1.61	5.248×10^{-4}
1.66	9.100×10^{-4}
1.67	1.202×10^{-3}
1.69	1.905×10^{-3}

$k_I = 1.770 \times 10^{-3}\ sec^{-1}$

$K_a = 6.0 \times 10^{-5}$

(j) α-Phenylsuccinamic acid

70.6°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.267	2.291×10^{-4}
1.588	6.310×10^{-4}
1.592	1.014×10^{-3}
1.715	2.884×10^{-3}
1.728	3.715×10^{-3}
1.771	7.943×10^{-3}

$k_I = 1.785 \times 10^{-4}$ sec^{-1}

$K_a = 4.2 \times 10^{-5}$

81.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.26	5.13×10^{-5}
2.80	1.585×10^{-4}
3.07	3.311×10^{-4}
4.06	1.230×10^{-3}
4.26	1.549×10^{-3}
4.49	2.570×10^{-3}
4.54	2.512×10^{-3}

$k_I = 4.727 \times 10^{-4}$ sec^{-1}

$K_a = 1.5 \times 10^{-4}$

92.8°

$10^3 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.725	9.55×10^{-5}
1.010	2.040×10^{-4}
1.290	6.166×10^{-4}
1.317	7.586×10^{-4}
1.380	1.023×10^{-3}

$k_I = 1.504 \times 10^{-3}$ sec^{-1}

$K_a = 1.0 \times 10^{-4}$

(k) Succinamic acid

69.2°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
6.14	1.751×10^{-4}
6.56	1.818×10^{-4}
7.56	7.242×10^{-4}
8.04	1.135×10^{-3}
8.20	2.291×10^{-3}

$k_I = 8.43 \times 10^{-5}$ sec^{-1}

$K_a = 6.5 \times 10^{-5}$

78.9°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.269	8.00×10^{-5}
2.000	4.601×10^{-4}
2.040	6.195×10^{-4}
2.230	2.300×10^{-3}
2.295	3.120×10^{-2}

$k_I = 2.275 \times 10^{-4}$ sec^{-1}

$K_a = 7.9 \times 10^{-5}$

91.5°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.80	3.01×10^{-5}
5.20	5.98×10^{-5}
6.11	1.201×10^{-4}
6.41	1.409×10^{-4}
6.84	2.032×10^{-4}
7.60	6.912×10^{-4}
7.93	5.471×10^{-3}
7.94	7.380×10^{-3}

$k_I = 7.835 \times 10^{-4}$ sec^{-1}

$K_a = 3.0 \times 10^{-5}$

(1) β-Methoxysuccinamic acid

70.7°

$10^5 k_{obs}$ (sec^{-1})	$a_{H_3O^+}$ (g. moles/l.)
6.35	9.77×10^{-5}
7.28	1.514×10^{-4}
8.40	3.020×10^{-4}
9.21	6.310×10^{-4}
9.26	8.128×10^{-4}

$k_I = 9.803 \times 10^{-5}\ sec^{-1}$

$K_a = 4.9 \times 10^{-5}$

78.3°

$10^4 k_{obs}$ (sec^{-1})	$a_{H_3O^+}$ (g. moles/l.)
1.38	1.995×10^{-4}
1.68	5.129×10^{-4}
1.80	7.943×10^{-4}
1.85	1.549×10^{-3}
1.86	2.630×10^{-3}

$k_I = 1.932 \times 10^{-4}\ sec^{-1}$

$K_a = 5.2 \times 10^{-5}$

93.1°

$10^4 k_{obs}$ (sec^{-1})	$a_{H_3O^+}$ (g. moles/l.)
6.08	1.660×10^{-4}
6.83	2.951×10^{-4}
7.43	6.166×10^{-4}
7.58	9.120×10^{-4}
7.72	1.380×10^{-3}
7.86	2.138×10^{-3}

$k_I = 8.04 \times 10^{-4}\ sec^{-1}$

$K_a = 5.3 \times 10^{-5}$

Reactions analysed by the distillation of ammonia.

(m) Isoasparagine

60.0°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.396	0.125
1.740	0.312
2.075	0.500
2.320	0.625

$k_I = 1.165 \times 10^{-5}\ sec^{-1}$

$k_{II} = 1.83 \times 10^{-5}\ l.mole^{-1}\ sec^{-1}$

78.9°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
9.60	0.153
10.81	0.306
11.90	0.459
13.20	0.612

$k_I = 8.47 \times 10^{-5}\ sec^{-1}$

$k_{II} = 7.58 \times 10^{-5}\ l.mole^{-5}\ sec^{-1}$

95.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
4.15	0.062
4.29	0.124
4.42	0.155
4.62	0.248
4.84	0.310

$k_I = 3.97 \times 10^{-4}\ sec^{-1}$

$k_{II} = 5.86 \times 10^{-4}\ l.mole^{-1}\ sec^{-1}$

$10^4 k_{obs}$* (sec^{-1})	[a] H_3O^+ (g. moles/l.)
1.15	2.480×10^{-4}
1.59	3.802×10^{-4}
2.13	6.990×10^{-4}
3.06	1.903×10^{-3}
3.70	6.415×10^{-3}

$k_I = 3.98 \times 10^{-4}\ sec^{-1}$

$K_a = 5.9 \times 10^{-4}$

---

* These results were obtained by the direct titration of ammonia.

(n) threo-α,β-Dimethoxysuccinamic acid

75.0°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
2.66	0.100
3.27	0.200
4.60	0.400
5.24	0.500

$k_I = 2.09 \times 10^{-5}\ sec^{-1}$

$k_{II} = 6.28 \times 10^{-5}\ l.mole^{-1}\ sec^{-1}$

85.2°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
7.87	0.100
9.80	0.200
13.70	0.400
15.70	0.500

$k_I = 5.93 \times 10^{-5}\ sec^{-1}$

$k_{II} = 1.93 \times 10^{-4}\ l.mole^{-1}\ sec^{-1}$

95.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.94	0.100
2.37	0.200
3.15	0.400
3.55	0.500

$k_I = 1.55 \times 10^{-4}\ sec^{-1}$

$k_{II} = 4.02 \times 10^{-4}\ l.mole^{-1}\ sec^{-1}$

(o) trans-Cyclohexane-1,2-dicarboxylic acid mono-amide

75.0°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.41	0.100
1.72	0.200
2.16	0.400
2.39	0.500

$k_I = 1.21 \times 10^{-5}\ sec^{-1}$

$k_{II} = 2.38 \times 10^{-5}\ l.mole^{-1}\ sec^{-1}$

85.0°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.88	0.100
4.45	0.200
5.62	0.400
6.06	0.500

$k_I = 3.30 \times 10^{-5}\ sec^{-1}$

$k_{II} = 5.82 \times 10^{-5}\ l.mole^{-1}\ sec^{-1}$

96.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.08	0.100
1.255	0.200
1.46	0.300
1.57	0.400
1.71	0.500

$k_I = 9.35 \times 10^{-5}\ sec^{-1}$

$k_{II} = 1.57 \times 10^{-4}\ l.mole^{-1}\ sec^{-1}$

(p) Asparagine

70.0°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
1.69	0.150
2.83	0.300
4.70	0.500
5.30	0.600

$k_I = 3.80 \times 10^{-6}\ sec^{-1}$

$k_{II} = 8.51 \times 10^{-5}\ l.mole^{-1}\ sec^{-1}$

80.0°

$10^5 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
3.68	0.150
6.55	0.300
9.95	0.500
12.15	0.600

$k_I = 1.05 \times 10^{-5}\ sec^{-1}$

$k_{II} = 1.80 \times 10^{-4}\ l.mole^{-1}\ sec^{-1}$

95.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.942	0.099
1.386	0.198
1.910	0.297
2.360	0.396
2.870	0.490

$k_I = 4.50 \times 10^{-5}\ sec^{-1}$

$k_{II} = 4.67 \times 10^{-4}\ l.mole^{-1}\ sec^{-1}$

(q) trans-Cyclopentane-1,2-dicarboxylic acid mono-amide

85.0°

$10^4 k_{obs}$ (sec^{-1})	H_3O^+ (g. ions/l.)
0.63	0.050
1.25	0.100
2.42	0.200
3.65	0.300
6.15	0.500

$k_I = 0$

$k_{II} = 1.226 \times 10^{-3}$ l.mole^{-1} sec^{-1}

75.0°

$H_3O^+ = 0.500$ g. ions/l. $\quad k_{obs} = 2.84 \times 10^{-4}$ sec^{-1}

$k_{II} = 5.69 \times 10^{-4}$ l.mole^{-1} sec^{-1}

65.0°

$H_3O^+ = 0.500$ g. ions/l. $\quad k_{obs} = 1.25 \times 10^{-4}$ sec^{-1}

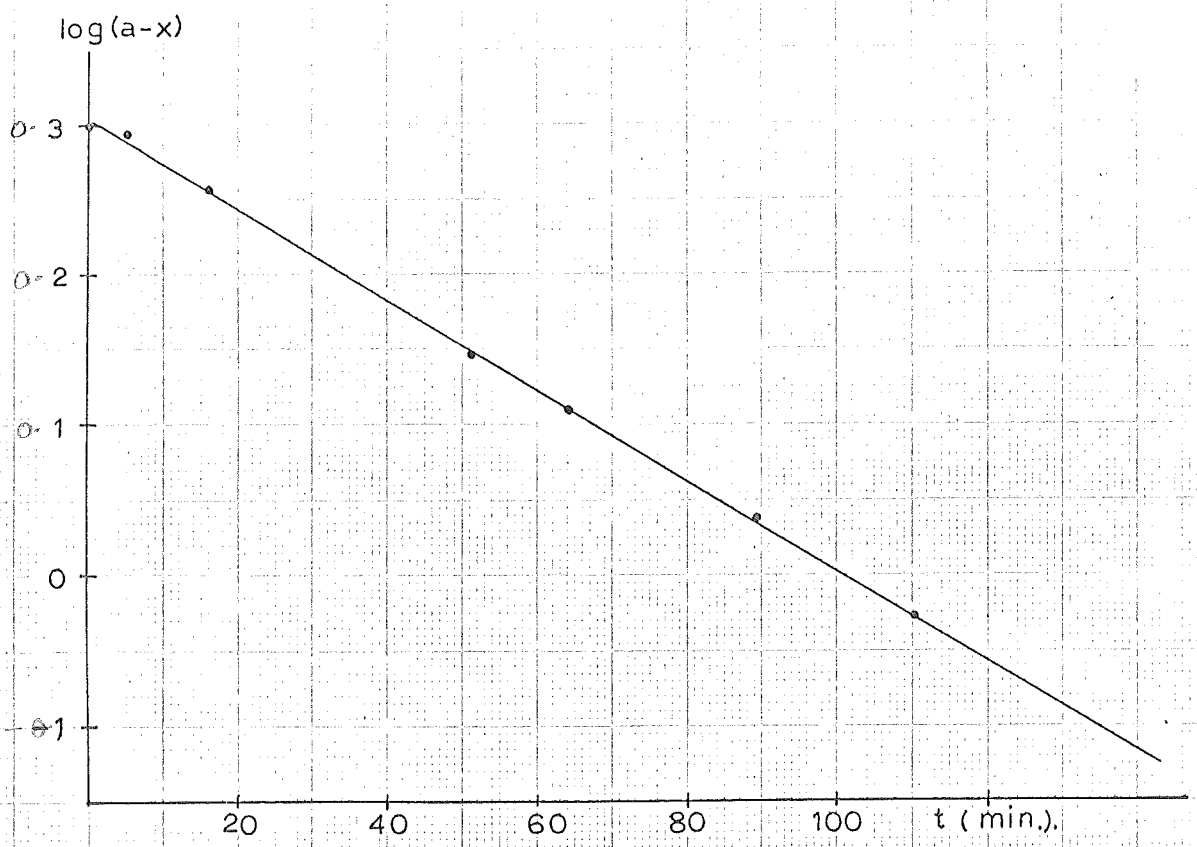
$k_{II} = 2.50 \times 10^{-4}$ l.mole^{-1} sec^{-1}

(r) α-Chlorosuccinamic acid

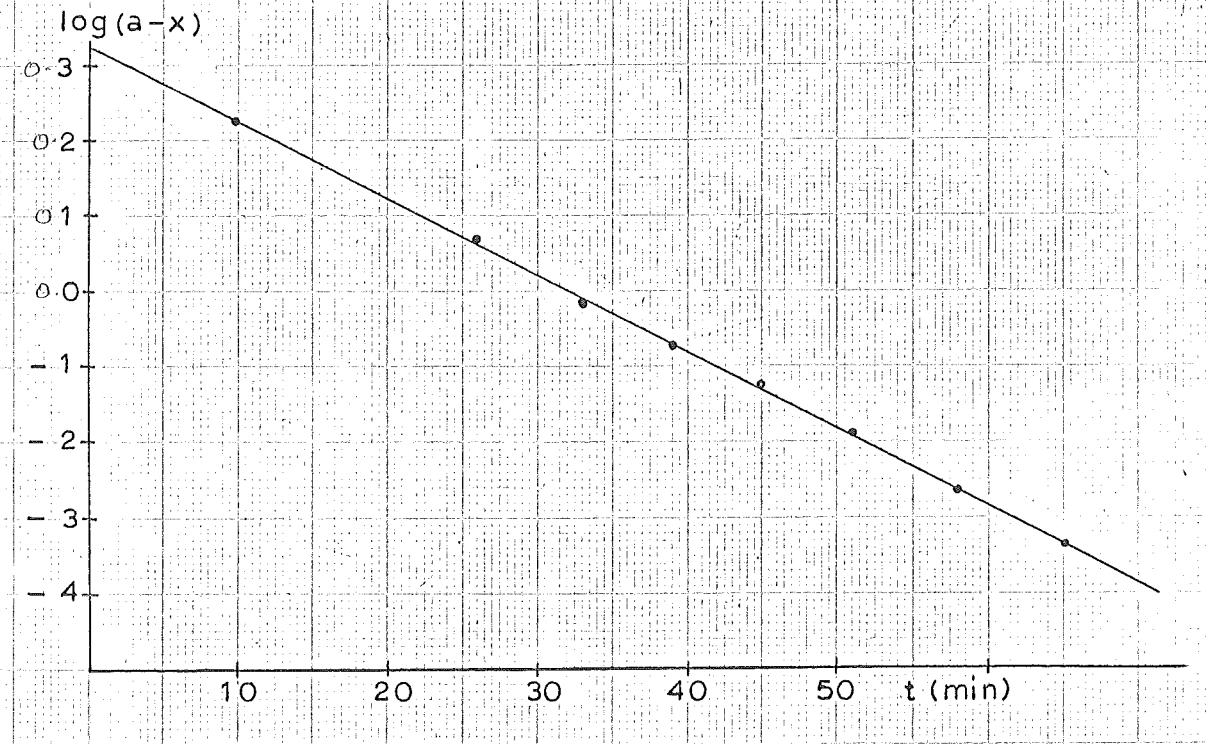
Holmberg has reported that when this acid was heated in water, or 0.5 M sulphuric acid, on a water bath, a mixture of α-chlorosuccinic acid, malic acid, and a

Fig III

Decomposition of α-Chlorosuccinamic Acid



Decomposition of α-Bromosuccinamic Acid



little fumaric acid was obtained. In the present work the rate of liberation of hydrogen chloride was determined before any attempts were made to measure the rate of hydrolysis of the amide.

Ten ml. samples of 0.02 M α-chlorosuccinamic acid were heated in stoppered sample tubes at 96.0°. The samples were withdrawn at regular intervals and the hydrogen chloride analysed by titration against silver nitrate in the presence of an excess of nitric acid. The plot of log(a - x) vs time shown in Fig.III is a straight line of slope ~~5.39~~ 4.98 x 10^{-5} sec^{-1} which corresponds to a first order rate constant of $k = $ ~~1.47~~ 1.15 x 10^{-4} sec^{-1}.

This rate is of a similar magnitude to that expected for the hydrolysis of the amide and therefore, the rate of amide hydrolysis of α-chlorosuccinamic acid could not be determined.

(s) <u>α-Bromosuccinamic acid</u>

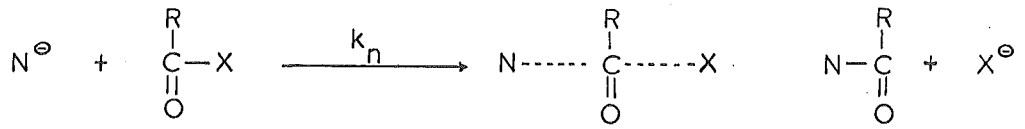
The rate of decomposition of α-bromosuccinamic acid was also measured in a manner identical with that used for α-chlorosuccinamic acid. A plot of log(a - x) vs time is shown in Fig. III. The corresponding first order rate constant, $k = 3.96 \times 10^{-4}$ sec^{-1} is also too fast to allow the rate of hydrolysis of the amide in α-bromosuccinamic acid to be determined.

DISCUSSION

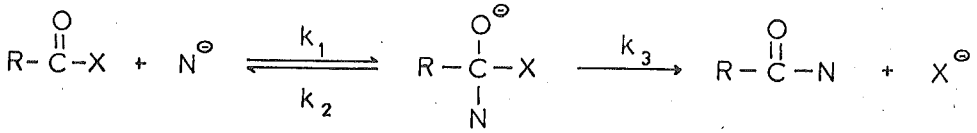
Theoretical Aspects

The attack of a nucleophile at a carbonyl carbon atom may occur by two mechanisms.

(a) A concerted displacement mechanism, S_N2.



(b) An addition-elimination mechanism



The most commonly observed mechanism for the acid or base catalysed hydrolysis of carboxylic acid derivatives is the addition-elimination mechanism involving the formation of a tetrahedral intermediate. The main evidence[2] distinguishing between these two mechanisms is the observation of concurrent isotopic oxygen-exchange during the hydrolysis. Such isotopic oxygen-exchange is inconsistent with an S_N2 mechanism

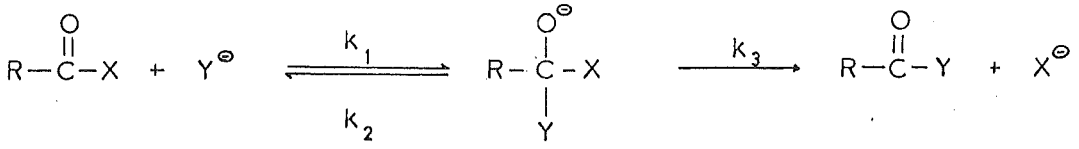
as the carbonyl oxygen is not involved in any reversible step. This concurrent carbonyl oxygen-exchange has been observed during the acid and base catalysed hydrolysis of amides, esters, anhydrides, and acid halides.[62] The existence of a number of stable addition complexes of carboxylic acid derivatives has been cited by Bender[62] as additional evidence for the formation of the tetrahedral intermediate.

During the intramolecular participation of a carboxyl group in the hydrolysis of a carboxylic acid derivative there is no direct way of determining whether a tetrahedral intermediate is formed, or whether the hydrolysis occurs by an S_N2 mechanism.

The following discussion concerns the structural effects on the rate of formation (k_1), and the partitioning (α) of the tetrahedral intermediate. However, for an S_N2 mechanism the structural effects on the rate of nucleophilic attack (k_n) will be similar to the structural effects on k_1 for the rate of nucleophilic attack leading to the formation of a tetrahedral intermediate.

The formation of an intermediate in the hydrolysis requires that the reaction takes place in two steps.

The reaction sequence is:



The kinetics of this process may be treated by the steady state approximation.

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_3} C$$

$$\frac{dB}{dt} = k_1 A - k_2 B - k_3 B = 0$$

$$-\frac{dA}{dt} = \frac{dC}{dt} = \frac{k_1 A}{(k_2 / k_3 + 1)} \qquad \ldots\ldots\ldots\ldots (3)$$

For substitution involving most carboxylic acid derivativies k_2 and k_3 are of comparable magnitude and equation (3) cannot be simplified. This, then, gives the overall rate constant as a function of two parameters: k_1, the rate constant for the formation of the tetrahedral intermediate, and k_2/k_3 (α), the partition coefficient of the intermediate.

The effect of structure on reactivity

The effect of substituents on the rate constant can be divided into the effects of the substituents on the

parameters k_1 and α.

An increase in the electron withdrawing character of R in R-COX would be expected to result in an increase in the rate of formation of the tetrahedral intermediate, k_1. This prediction is supported by the position of the equilibrium of the addition of methoxide ion to the ethyl fluoracetates[63] shown in Table IV. A similar trend is observed for the base catalysed hydrolysis of the ethyl chloroacetates.[63]

Table IV

Ester	Relative k_0^{OH} (ethyl chloroacetates)	Addn. of $MeO^{\ominus}$ (ethyl fluoroacetates)
acetate	1	0%
monohaloacetate	761	26%
dihaloacetate	16,000	77%
trihaloacetate	100,000	99%

This increase in the rate of solvolysis of carboxylic acid derivatives with the increase in the -I character of the substituent has been well demonstrated[64] for a large number of aromatic carboxylic acid derivatives by Hammett's linear free energy relationship.

$$\log k_x/k_o = \rho\sigma$$

The relative insensitivity of amides and esters to the electronic effects of their substituents during acid catalysed hydrolysis at first sight appears anomalous. Edwards,[65] however, suggested that acidic hydrolysis of amides and esters proceeded via a pre-equilibrium protonation followed by rate limiting attack of water on the conjugate acid of the substrate.

$$H^+ + R\text{-}CONH_2 \rightleftharpoons R\text{-}CONH_3^+ \xrightarrow{H_2O} R\text{-}CO_2H + NH_3 + H^+$$

Protonation of amides should be facilitated by electron-releasing substituents (ρ_1 -ve), whereas nucleophilic attack by water on the protonated substrate should be enhanced by electron-withdrawing substituents (ρ_2 +ve). At acidities where the substrate is essentially non-protonated the overall sensitivity of the hydrolysis to electronic effects (ρ_o) should equal the sum of the sensitivities for protonation and nucleophilic attack.

$$\rho_o = (\rho_1 + \rho_2)$$

Edward[65] has determined ρ_1 for substituted benzamides from the pK_a' for the amide protonation, and ρ_2 for the hydrolysis of benzamides in concentrated perchloric acid. The experimentally determined ρ_o (= 0.118) is in good agreement with the sum $\rho_1 + \rho_2$ (= -1.30 x 1.27 = -0.03).

Furthermore, the value for the acidic hydrolysis of benzamides is comparable to the ρ(= 1.06) obtained for the alkaline hydrolysis of benzamides.

It was originally assumed that structural changed in R would have no effect on α. In the case of p-substituted methyl benzoates Bender[66] has shown that the ratio k_h/k_{ex} is dependent on the substituents.

Table V[66]

Substituents of X--CO_2 Me	k_h/k_{ex}	$10^2 k_h$ ($l.mole^{-1} sec^{-1}$)
NH_2 -	30	1.14
CH_3 -	11	11.1
H-	5.2	23.2
Cl-	6.3	68
NO_2 -	2.8	700

He explained this variation in k_h/k_{ex} as being due to a kinetically significant proton transfer in the formation of the intermediary un-ionized ester hydrate, R-C(OH)$_2$X. The effect on α, however, is significant when compared with the effect on k_1. Bender has shown that by ignoring the variation in α in this case, a

satisfactory Hammett plot may be obtained with $\rho = 1.93$.

$$\text{i.e.} \quad \log k_h/k_h^o \simeq \log k_1/k_1^o + \text{constant}$$

Taft[67] has successfully separated the steric effects from the electronic effects of the substituents and has shown that for intermolecularly catalysed solvolysis of aliphatic carboxylic acid derivatives, and ortho-substituted benzoic acid derivatives, the rate of solvolysis decreases with the increasing bulk of the substituent. This can be explained by increasing steric hindrance to the attack of the nucleophilic species.

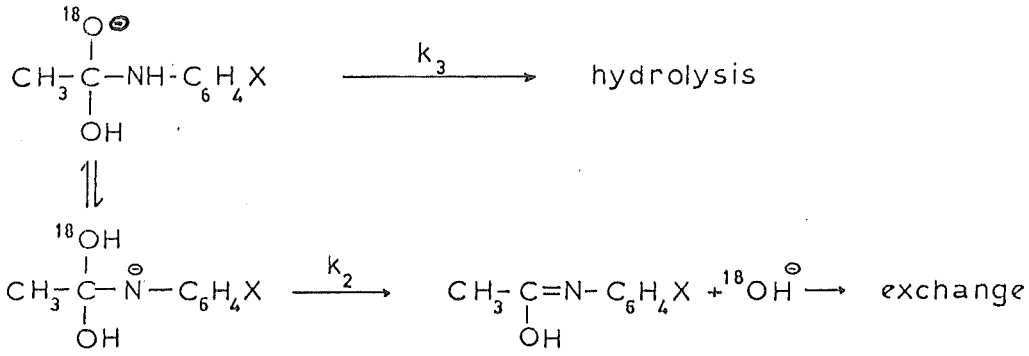
In intramolecularly catalysed solvolysis of carboxylic acid derivatives the rate of solvolysis generally increases with the increasing bulk of the substituents. This has been explained by Bruice[20,68] as being due to the increasing steric constraint of the participating groups.

In both inter- and intramolecularly catalysed solvolyses the steric effect of the substituents would be expected to affect only k_1, the rate of formation of the tetrahedral intermediate. The effect on α, the partition coefficient, would be expected to be negligible unless the ratio of the size of the leaving group to the

size of the nucleophile was large. In this case, α would be expected to decrease. Support for this prediction may be drawn from the failure to observe concurrent isotopic oxygen-exchange with the hydrolysis of substituted-benzyl benzoates.[69]

The effect of structural changes in X of R-COX have not been as extensively investigated as the effects of structural changes in R.

By analogy with the effect of the -I substituents of R, an increase in k_1 would be expected with an increase in the electron-withdrawing character of X. This has been verified by positive slopes in the Hammett plots of the hydrolyses of substituted-phenyl acetates[70] and benzoates.[71] The alkaline hydrolysis of p-substituted acetanilides,[72] however, shows little dependance on the electronic effects of its substituents. Bender and Thomas have measured the rate of isotopic oxygen-exchange during the hydrolysis and have obtained Hammett plots for σ vs log k_1 and σ vs log α with slopes $\rho = +1.0$ and $\rho = -1.0$ respectively. A positive ρ was expected for the partitioning of the intermediate as -I substituents would be expected to stabilize the anionic leaving group. Bender explained this anomaly by the following mechanism.



The negatively charged nitrogen is stabilized by the -I substituents and the exchange reaction is then more favoured than the hydrolysis reaction.

Resonance effects of the groups R and X must also be considered. Increased resonance interaction between the carbonyl group and R or X will increase the stability of the ground state relative to the transition state and, consequently will decrease k_1. This is demonstrated by the relative rates of hydrolysis of amides, esters, anhydrides, and acid chlorides. The reactivity increases and the resonance interaction decreases in the above order.[62] Similarly benzoic acid derivatives hydrolyse much more slowly than the corresponding aliphatic acid derivatives.

As pointed out above, the overall rate constant of the forward reaction of R-COX + Y will depend on k_1 and α.

k_1 is clearly dependent on the nucleophilicity of the attacking species. Attempts to relate structure to nucleophilicity have so far not been entirely successful. Swain,[73] and Edwards[74] have proposed linear free energy relationships to correlate the rate constants of nucleophilic substitution reactions. These equations have met with only limited success in that the parameters expressing nucleophilicity are only constant for a group of very similar nucleophiles. Edwards' equation, however, indicates that the basicity of the attacking nucleophile is the main factor in determining the relative reactivity in the groups of nucleophiles towards carbonyl carbon atoms.

Bruice[75] has demonstrated this relationship of basicity to nucleophilicity by Brönstead-type plots of log k vs pK_a' for the hydrolysis of p-nitro-phenyl acetate catalysed by restricted families of nucleophiles. Positive slopes in these plots indicate that the hydrolysis is favoured by electron-donating substituents in the nucleophile.

The effect on α of the structural changes in the nucleophile depends on the relative stabilities of the nucleophiles X and Y as well as those of the carboxylic acid derivatives R-COY and R-COX. Electron-donating

substituents in Y will, therefore, lead to a decrease in α by decreasing the stability of Y and increasing the stability of R-COY.

A summary of the electronic effects of the substituents in R, X, and Y are given in Table VI.

Table VI[62]

Increase in -I Character of Substituent in	Effect on k_1 (or k_n)	Effect on α	Total Effect
R	increase	increase	increase
X	increase	decrease	increase
Y	decrease	increase	decrease

Table VII

Substituents of Succinamic Acid	k_I at 70.0° (sec^{-1})	k_I/k_I°
<u>cis</u>-cyclopentane	1.86×10^{-2}	204.0
<u>cis</u>-cyclohexane	4.32×10^{-3}	47.4
β,β-dimethyl	3.10×10^{-3}	42.7
α,α-dimethyl	3.89×10^{-3}	40.6
<u>threo</u>-α,β-dimethyl	3.74×10^{-3}	34.0
<u>erythro</u>-α,β-dimethyl	1.05×10^{-3}	11.5
β-methyl	3.59×10^{-4}	3.94
α-methyl	2.75×10^{-4}	3.01
β-phenyl	1.82×10^{-4}	1.30
α-phenyl	1.55×10^{-4}	1.26
H (succinamic)	9.12×10^{-5}	1.00
β-methoxy	8.91×10^{-5}	0.98
β-amino (NH_3^+-)	3.43×10^{-5}	0.37
<u>threo</u>-α,β-dimethoxy	1.23×10^{-5}	0.135
<u>trans</u>-cyclohexane	7.16×10^{-6}	0.078
α-amino (NH_3^+-)	3.80×10^{-6}	0.031
<u>trans</u>-cyclopentane	0.0	0.000
maleamic acid[77]	4.09×10^{-3}	44.8
phthalamic acid[76]	1.78×10^{-3}	19.3
L-leucylasparagine[10]	3.39×10^{-5}	0.37
glycyl-L-asparagine[10]	2.62×10^{-5}	0.29

Summary of Results

The experimental data for the unimolecular rate constants are shown in Fig. IV. as a plot of log k_I vs 1/T. The rate constants at 70.0° in Table VII were obtained from this graph by extrapolation. The unimolecular rate constants at 70.0° for the hydrolysis of phthalamic acid,[76] maleamic acid,[77]* glycyl-L-asparagine,[10] and L-leucylasparagine[10] were obtained by extrapolation of the relevant data reported in the literature.

The thermodynamic functions given in Table VIII were obtained from the values of the rate constants at 70.0° given in Table VII and the slopes of the plots of log k_I vs 1/T in Fig. IV by the equations:

$$\Delta G^{\ddagger} = 2.303\ RT \log (kT/hk_I)$$

$$\Delta H^{\ddagger} = E_a - RT$$

$$\Delta S^{\ddagger} = (\Delta G^{\ddagger} - \Delta H^{\ddagger})/T$$

E_a, Arrhenius' energy of activation was obtained from Fig. III by application of Arrhenius'

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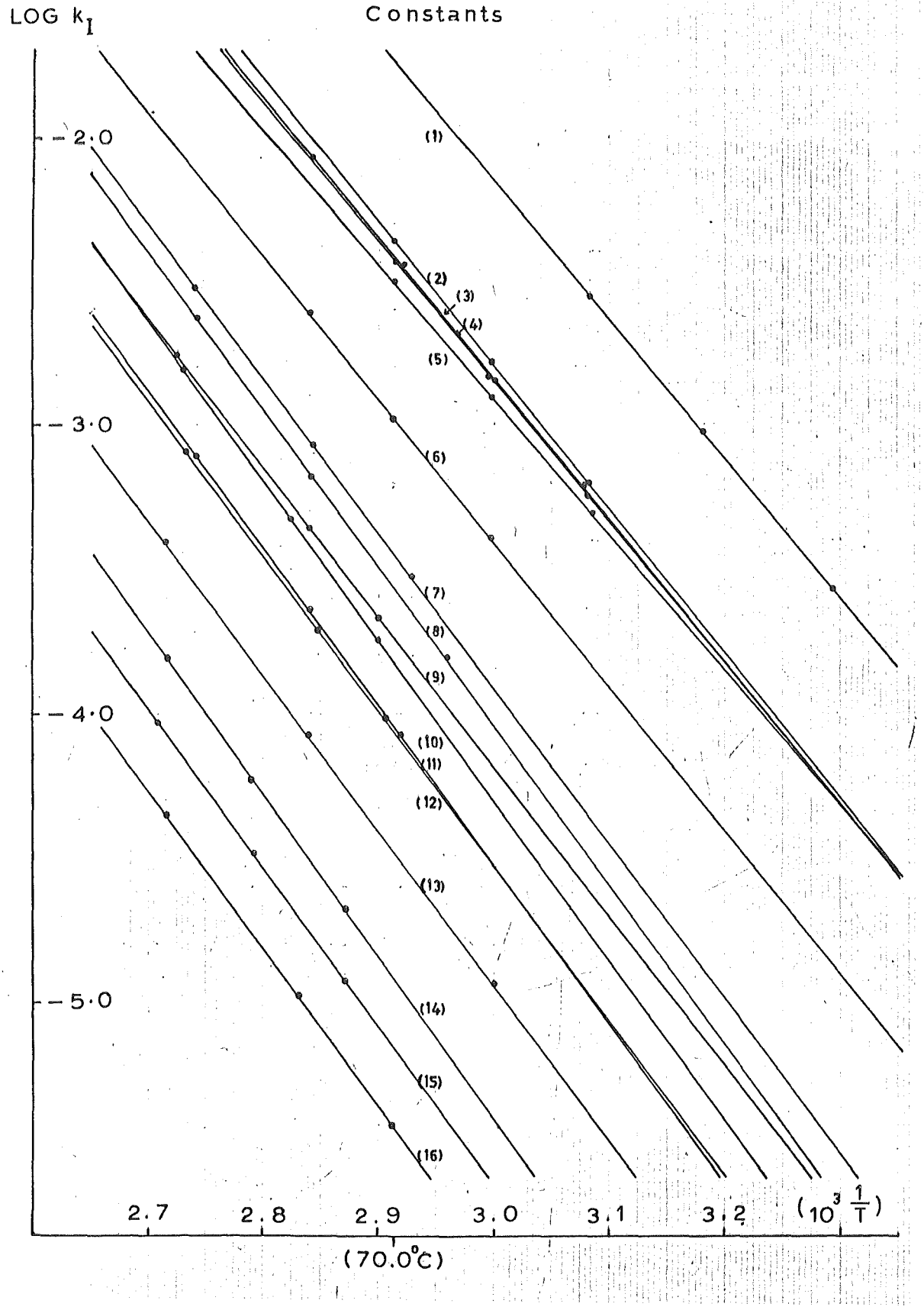
* Only the rate at 65° was reported. The rate at 70° was estimated from the equation $\Delta G^{\ddagger} = 2.303\ RT \log (kT/hk_I)$.

Fig IV

Temperature Dependence of the Unimolecular Rate Constants



Key to Fig. IV

(1) cis-Cyclopentane-1,2-dicarboxylic acid mono-amide

(2) cis-Cyclohexane-1,2-dicarboxylic acid mono-amide

(3) β,β-Dimethylsuccinamic acid

(4) α,α-Dimethylsuccinamic acid

(5) threo-α,β-Dimethylsuccinamic acid

(6) erythro-α,β-Dimethylsuccinamic acid

(7) β-Methylsuccinamic acid

(8) α-Methylsuccinamic acid

(9) β-Phenylsuccinamic acid

(10) α-Phenylsuccinamic acid

(11) Succinamic acid

(12) β-Methoxysuccinamic acid

(13) Isoasparagine

(14) threo-α,β-Dimethoxysuccinamic acid

(15) trans-Cyclohexane-1,2-dicarboxylic acid mono-amide

(16) Asparagine

Table VIII

Thermodynamic Functions for Unimolecular Rates at 70.0°

Substituents of Succinamic Acid	$\Delta G^{\ddagger}$ k.cals/mole	$\Delta H^{\ddagger}$ k.cals/mole	$\Delta S^{\ddagger}$ e.u.
cis-cyclopentane	22.9	21.5	- 4.2
cis-cyclohexane	23.9	21.7	- 6.3
β,β-dimethyl	24.0	21.3	- 7.7
α,α-dimethyl	24.0	21.5	- 7.1
threo-α,β-dimethyl	24.1	20.3	-11.0
erythro-α,β-dimethyl	24.9	21.6	- 9.4
β-methyl	25.6	23.2	- 6.9
α-methyl	25.8	23.8	- 5.7
β-phenyl	26.0	23.0	- 8.8
α-phenyl	26.2	23.6	- 7.3
H (succinamic)	26.5	24.1	- 7.0
β-methoxy	26.5	23.3	- 9.5
β-amino (NH_3^+-)	27.2	23.6	-10.4
threo-α,β-dimethoxy	27.9	24.5	-10.0
trans-cyclohexane	28.3	24.2	-11.9
α-amino (NH_3^+-)	28.7	24.9	-10.9
phthalamic acid[76]	24.5	20.7	-12.2
L-leucylasparagine[10]	27.2	22.1	-14.9
glycyl-L-asparagine[10]	27.4	23.6	-11.1

equation:

$$2.303 \log k_I = -E_a/RT + \text{constant}$$

The second order rate constants were also obtained for the hydrolysis of trans-cyclopentane--1,2-dicarboxylic acid mono-amide, asparagine, threo--α,β-dimethoxysuccinamic acid, isoasparagine, and trans-cyclohexane-1,2-dicarboxylic acid mono-amide. Their thermodynamic functions at 70.0° are tabulated in Table IX.

Table IX

Thermodynamic Functions for Bimolecular Rates at 70.0°

Substituents of Succinamic Acid	$\Delta G^{\ddagger}$ k.cals/mole	$\Delta H^{\ddagger}$ k.cals/mole	$\Delta S^{\ddagger}$ e.u.
trans-cyclopentane	25.8	18.3	-22.1
α-amino (NH_3^+-)	26.6	18.0	-25.0
threo-α,β-dimethoxy	26.9	20.3	-19.0
β-amino (NH_3^+-)	27.0	18.2	-25.6
trans-cyclohexane	27.7	19.6	-23.5

Accuracy of Results

As has been stated previously (p.58) the reproducibility of the two methods of analysis is of the order of $\pm$ 1-2%. However, systematic errors may be present for which no allowance has been made. If these are estimated to be of the order of $\pm$ 5% the corresponding probable errors in the thermodynamic functions are $\pm$0.05k.cals.mole^{-1}, $\pm$0.4 k cals.mole^{-1}, $\pm$1.3e.u. in the changes in the free energy, enthalpy, and entropy of activation respectively.

The unimolecular rate of hydrolysis of succinamic acid has been reported[77] previously and the value of $k_I = 5.09 \times 10^{-5}$ sec^{-1} at 69.3° is in disagreement with the value of $k_I = 8.43 \times 10^{-5}$ sec^{-1} obtained at 69.2° in the present work. Higauchi[78] has measured the pH-rate profile at 69.3° of succinamic acid. The plateau observed at pH 2 corresponds to a rate of $k_I = 8.1 \times 10^{-5}$ sec^{-1} which is in good agreement with figures obtained in the present work.

INTERPRETATION OF RESULTS

General

In this section the following symbols have been used, and are defined as follows:-

k_I The rate constant of the intramolecularly catalysed hydrolysis.

k_1 The rate constant for nucleophilic attack of the carbonyl carbon atom leading to the formation of a tetrahedral intermediate.

k_2 The rate constant for the collapse of the tetrahedral intermediate to give the initial substrate.

k_3 The rate constant for the collapse of the tetrahedral intermediate to give the hydrolysis products.

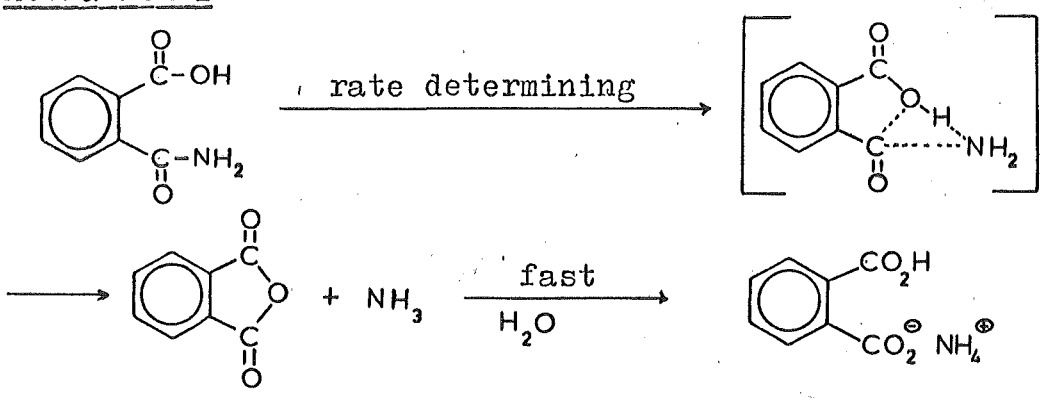
k_n The rate constant for nucleophilic attack of the carbonyl carbon atom in an S_N2 mechanism.

K The coefficient of the mole fraction of the substrate in the reactive conformation (see p.98).

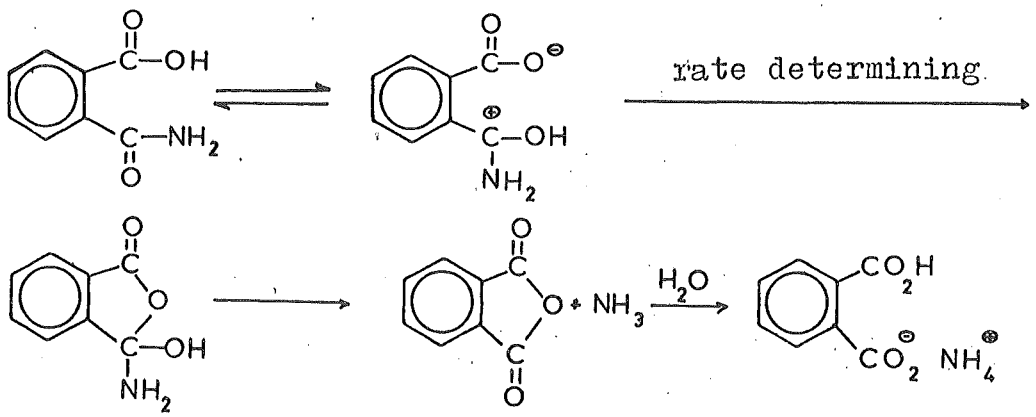
Mechanisms Involving Intramolecular Participation of Carboxyl Groups in the Hydrolysis of Amides

Two mechanisms were proposed by Bender[18] for the hydrolysis of phthalamic acid.

Mechanism I

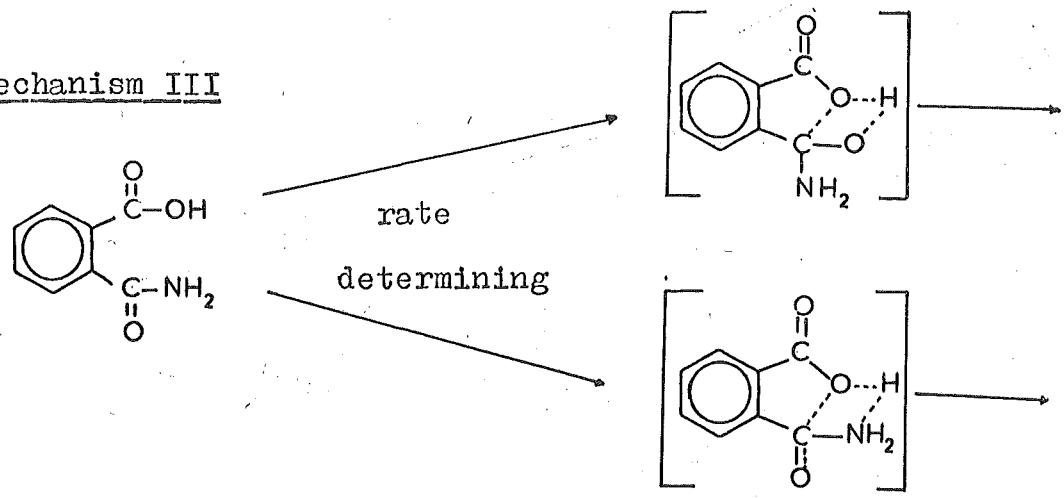


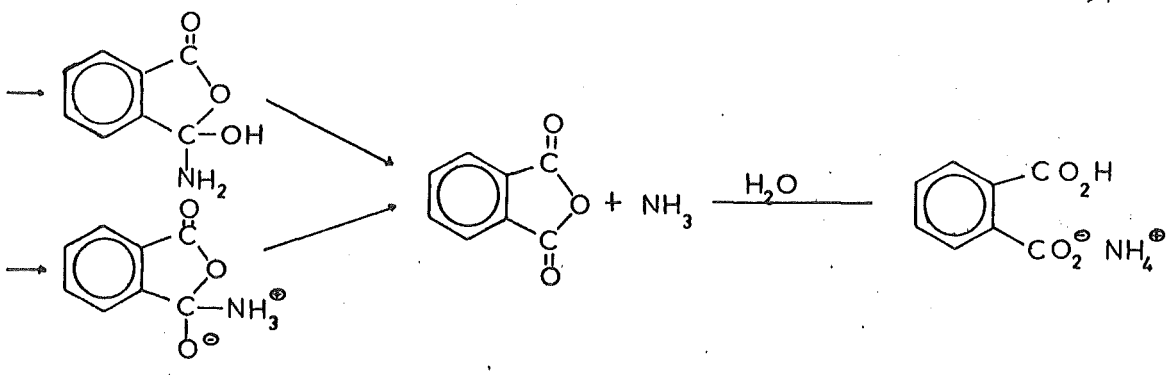
Mechanism II



A third mechanism is also possible in which bond formation between the acidic proton and the amide group occurs in the transition state.

Mechanism III





Mechanism III is an extension of mechanism II where nucleophilic attack on the carboxyl group accompanies the transfer of the proton. Considerable controversy[86] has existed as to whether the protonation of the nitrogen or oxygen is kinetically more significant in the hydrolysis of amides and, therefore, no distinction between the two paths in mechanism III can be made on this basis.

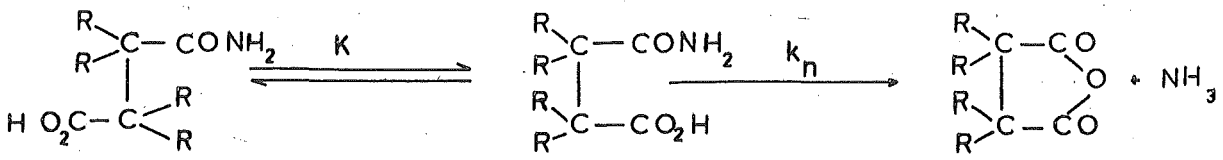
The three mechanisms are in agreement with all the observed experimental data for the hydrolysis of amides, anilides, and esters which are catalysed by the undissociated carboxyl groups. The mechanisms II and III give rise to a tetrahedral intermediate after the rate determining transition state. Mechanism I, however, resembles a direct S_N2 displacement reaction and no tetrahedral intermediate would be observed. If, however, the carbon-nitrogen bond was not broken in the rate determining step then mechanism I becomes identical with III where protonation of the nitrogen is considered kinetically important.

Although all three mechanisms are kinetically indistinguishable, mechanism II has not received much support and, therefore, only the mechanisms I and III will be considered in the discussion in this section.

Steric Effects of the Substituents

Before intramolecular catalysis can occur the two reacting groups of the molecule must be brought into close proximity. In the case of cis-cyclopentane-1,2--dicarboxylic acid mono-amide, maleamic acid, and phthalamic acid the groups are already held in this conformation by the rigidity of the molecule. However, for succinamic acid, and substituted succinamic acids free rotation may occur about the C-C bond. This allows the succinamic acids to exist as an equilibrium mixture of two conformers where the reacting groups are eclipsed or anti to each other. The rate expressions for the mechanisms I and III must now incorporate K, the coefficient of the mole fraction in the reactive conformation.

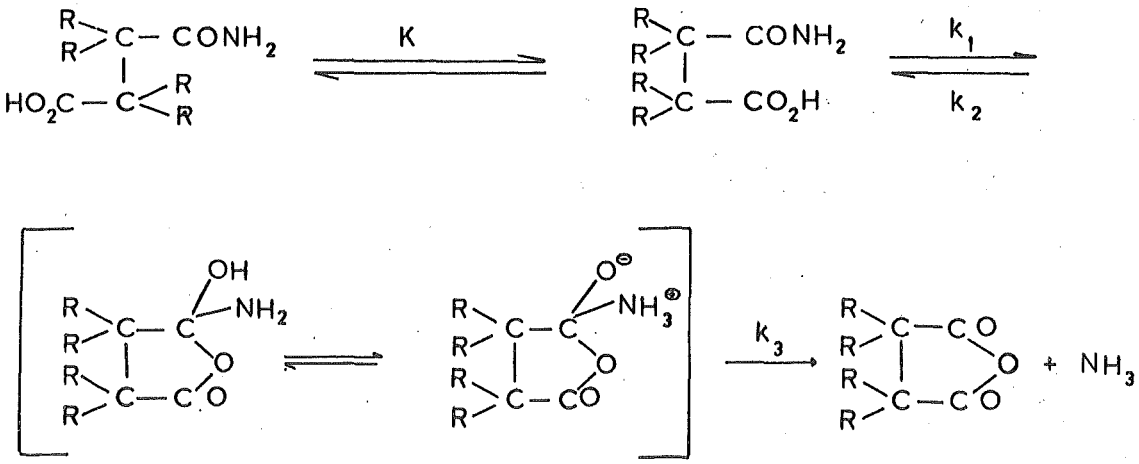
For the S_N2 mechanism:



$$k_I = K k_n$$

Where k_I is the unimolecular rate of hydrolysis and k_n is the rate determining nucleophilic attack.

For the addition-elimination mechanisms involving the formation of a tetrahedral intermediate:



$$k_I = K k_1/(\alpha + 1) \qquad \alpha = k_2/k_3$$

In this case the rate is dependent on both the rate of nucleophilic attack, k_1, and the partitioning of the intermediate, α.

By increasing the bulk of R the non-bonded repulsions between the carboxyl and amide groups, and R, are maximized and the concentration of the eclipsed conformer is increased. The resulting increase in the rate of hydrolysis with substitution is therefore due to a decrease in the probability of the unprofitable rotamer distribution in which the reacting groups lie away from each other.

The influence of alkyl substituents on the

rotamer distribution of alkyl succinic acids has been known for some time.[79] With the introduction of alkyl groups into the α and α' positions of succinic acid the first dissociation constant is markedly increased and the second dissociation constant decreased. This variation of log K_{a_1}/K_{a_2} with substitution has been correlated with the interprotonic distance (r) between the carboxyl groups of the acids by Westheimer and Shookoff.[80]

$$\log K_{a_1}/K_{a_2} = \frac{e^2}{2.303\ kTD_e r}$$

Dibasic Acid	ΔpK_a	r (Å)
Succinic acid	0.84	5.75
meso-α,α'-dimethyl	1.54	5.35
(±)-α,α'-dimethyl-	1.66	5.30
meso-α,α'-diethyl-	2.23	5.10
(±)-α,α'-diethyl-	2.49	5.00
tetramethyl-	4.19	4.80

An increase in the rate of ring formation of 2-substituted-4-bromobutylamines with the increase in the bulk of the substituent has been observed by Brown and Gulick.[79]

Bruice, Bradbury,[22] and Pandit[20] have proposed

Table X

The relative rates of hydrolysis of alkyl-substituted-succinic acid mono-p-methoxy esters, mono-amides, and mono-anilides

Substituents of Succinic Acid	k_I/k_I^o (esters)[20]	k_I/k_I^o (amides)	k_I/k_I^o (anilides)[78]
trans-cyclohexane	-	0.078	-
H (succinic acid)	1	1	1
α-phenyl	-	1.26	-
β-phenyl	-	1.30	-
α-methyl	-	3.0	2.6
β-methyl	-	3.9	-
erythro-α,β-dimethyl	-	11.5	5.6
phthalic	-	19.3	-
threo-α,β-dimethyl	-	34.0	23.4
α,α-dimethyl	31.7	40.6	10.6
β,β-dimethyl	-	42.7	-
maleic	43.5	44.8	-
cis-cyclohexane	-	47.4	-
cis-cyclopentane	-	204	-
3,4-endoxo-Δ^4- -tetrahydrophthalic	230	-	-
trimethyl	-	-	130
tetramethyl	-	-	383

that similar steric effects are operative in the intramolecularly catalysed hydrolysis of mono-p-bromophenyl esters of substituted glutaric and succinic acids. They have shown by a plot of statistically corrected pK'_a values vs log k_I/k_I^o for β,β-substituted glutaric acids that the hydrolytic rate constants exhibit a similar dependance on the bulk effect of the substituents to the ionization of the substituted glutaric acids. The effect of rotamer distribution on k_I has been further demonstrated by Bruice and Pandit.[20] By freezing out rotation about a single C-C bond they observed a 230 fold rate increase for the hydrolysis of mono-p--methoxyphenyl esters of glutaric acid, succinic acid, and 3,6-endoxo-Δ^4-tetrahydrophthalic acid.

A similar effect has been observed in the present work for the hydrolysis of alkylsuccinamic acids. Furthermore, it can be seen from Table X that the magnitude of the bulk effect of the substituents is similar for both ester and amide hydrolysis.

The electronic effect of alkyl substituents as correlated by Taft's[67] σ^* constants is relatively small and could only explain small changes in the rate of hydrolysis. The observed magnitude of the variation

of the rate constants, therefore, must be attributed to steric factors.

This is in agreement with the findings of Bruice and Bradbury[22] who showed, that by ignoring any electronic effects of the alkyl substituents in the hydrolysis of mono-p-bromophenyl esters of β-substituted glutaric acids, a plot of log k_I/k_I^o vs E_s (Taft's steric substituent constant) gave a reasonably straight line. The steric reaction constant ($\delta = -0.83$) was opposite in sign and equal in magnitude to the steric reaction constant obtained from the hydrolysis of β-substituted glutaric anhydrides[81] indicating that the increasing bulk has an accelerating effect on anhydride formation and a retarding effect on the ring opening.

For cis-cyclopentane-1,2-dicarboxylic acid mono-amide, maleamic acid, phthalamic acid, and the esters of 3,6-endoxo-Δ^4-tetrahydrophthalic acid, and maleic acid the reactant groups are held rigidly in an eclipsed conformation. The rate of nucleophilic attack of the participating carboxyl group or carboxylate anion (k_1 or k_n) should, therefore, be expected to be at a maximum.

The five fold difference in the rates of

hydrolysis of the unsaturated maleate ester and the 3,6-endoxo-Δ^4-tetrahydrophthalate has been attributed[70] to the greater separation of the reactant groups in the maleate ester due to the sp^2 character of the α,α' carbon atoms. A similar explanation would also be valid in accounting for the differences in the rate of hydrolysis of cis-cyclopentane-1,2-dicarboxylic acid mono-amide and the rates of hydrolysis of maleamic and phthalamic acids, but it fails to explain the difference observed in the rates of hydrolysis of the last two amic-acids. This difference in the rates is probably associated with the loss of the ground state resonance interactions of the amide group with the benzene ring or double bond, in the transition state. As more extensive resonance interactions are possible in phthalamic acid, its rate of hydrolysis would be expected to be correspondingly lower than the rate of hydrolysis of the maleamic acid.

It may be anticipated that the acceleration of the rate due to the continued forcing of the reactant groups into closer proximity will change gradually from a ground-state phenomenon, attributed to decreases in the extended rotamer populations, to a transition state phenomenon, attributed to the release of strain with the formation of the intermediate anhydride. A possible

example of this is found in the hydrolysis of methyl hydrogen 2,3-di-(t-butyl)succinate[15] where the intermediate anhydride is the stable product of the pseudo hydrolysis. The transition of these two effects, however, is difficult to predict and it is possible that part of the acceleration of the rate of hydrolysis of cis-cyclopentane-1,2-dicarboxylic acid mono-amide is due to relief of steric strain in the transition state.

The remarkable difference in the rates of hydrolysis for cis- and trans-cyclohexane-1,2-dicarboxylic acid mono-amides can be explained as being due to the strain involved in the formation of a five-membered ring in the trans-cyclohexane-1,2-dicarboxylic anhydride. If an S_N2 mechanism is operative then this 600 fold difference in the rates is due to the differences in the rates of nucleophilic attack of the carboxyl group in the two amic-acids. If an addition-elimination mechanism is operating then a considerable amount of the difference in the rates would be due to differences in α.

The most stable conformer for the trans-amic--acid is a "chair-shaped" cyclohexane ring with the amide and carboxyl groups occupying equatorial positions. In this conformation both the reactant groups are in reasonably close proximity, but the nucleophilic attack

of the carboxyl group (k_1, or k_n) on the carbonyl carbon atom of the amide group results in the formation of a strained five-membered ring. This strain, which is clearly demonstrated by means of models, would be expected to greatly increase the energy barrier involved in the nucleophilic attack. If a tetrahedral intermediate were formed during the hydrolysis, then its collapse would be expected to occur mainly by the path k_2 as this would relieve the strain of the five-membered ring and increase the resonance in the products.

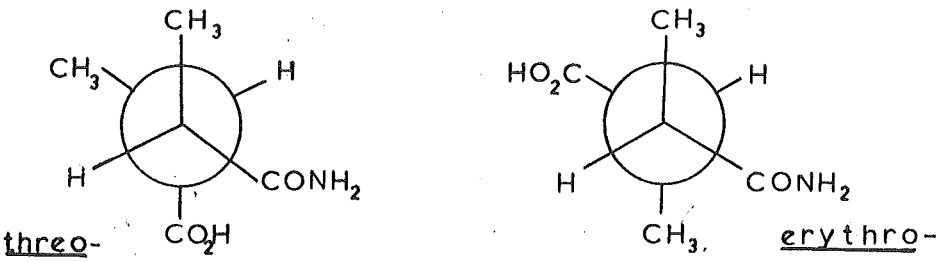
For cis-cyclohexane-1,2-dicarboxylic acid mono-amide the most stable conformer is a skew boat form. Here again the two reactant groups are in reasonably close proximity but the formation of the five-membered ring is not accompanied by the production of steric strain.

The three fold difference in the rate of hydrolysis of threo- and erythro-α,β-dimethylsuccinamic acids is similar to the differences in the rates for the hydrolysis of threo- and erythro-α,β-dimethylsuccinanilic acids observed by Higuchi.[78] He described this difference as a direct measure of the two systems' ability to cyclize. It becomes apparent by the inspection of models, however, that if the hydrolysis occurs by an addition-elimination mechanism then this

generalization is only part of the explanation. The differences in the rates would also be reflected in the relative stabilities of the amic-acids and their intermediate anhydrides.

The ability of these two systems to cyclize is dependent on the value of k_1K or k_nK. The magnitude of k_1 or k_n would be expected to be similar for both the threo- and erythro- isomers, but K will be influenced by the variation in the non-bonded repulsions of the substituents. The most stable conformers of the amic--acids are shown in Fig. V.

Fig. V.

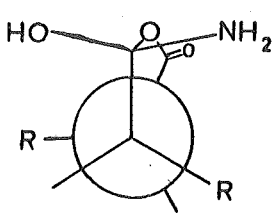


Because the gauche methyl-carboxyl and methyl--amide interactions are less important than the gauche methyl-methyl and amide-carboxyl interactions the erythro- isomer is the more stable of the two. This effect has also been observed for the corresponding dicarboxylic acids where the meso- isomer is the more stable. It is evident then, that the population of the conformation where the reactant groups are gauche

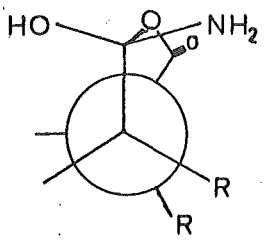
or eclipsed is higher for the threo- isomer than for the erythro- isomer. This results in an increase in the value of K, the coefficient of the mole fraction in the reactive conformation, and gives rise to a corresponding increase in the rate of hydrolysis.

The collapse of a tetrahedral intermediate would also effect the observed rate constant. On examination of the conformation of the tetrahedral intermediates,

Fig. VI



threo-*



erythro-

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* This conformer is shown slightly skew for ease of pictorial representation. The most stable arrangement is obtained when the hydrogen and methyl groups are eclipsed and the five-membered ring is planer.

it can be seen that the threo- isomer is the more stable, as the interactions between the eclipsed hydrogens and methyl groups are much less severe than the interaction between the eclipsed methyl groups in the erythro- isomer. Furthermore, collapse of these intermediates via the path k_3 to yield the cyclic anhydrides will not relieve the strain associated with these interactions, and correspondingly the meso- anhydride will remain the less stable. The collapse of the intermediate via the path k_2 to yield the initial amic-acids, however, relieves the strain associated with the eclipsing of the methyl groups in the erythro- intermediate.

A comparison of the ground state conformations of the products and substrates, and the conformation of the tetrahedral intermediates shows that the collapse of the tetrahedral intermediate will be favoured by the path k_3 for the threo- isomer but by the path k_2 for the erythro- isomer. The overall effect on k_I in this case, therefore, would be found in both K and α for

$$k_I = k_1 K/(\alpha + 1))$$

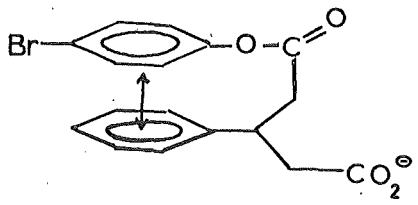
Since the difference in the rates of hydrolysis of the two isomers is only three fold the contribution from

α to the rate constant must be only of secondary importance.

The magnitudes of the rates of hydrolysis of α-phenylsuccinamic acid, and β-phenylsuccinamic acid are much lower than would be anticipated from the known size of the phenyl group, and the effect of the bulk of the substituent on the intramolecularly catalysed hydrolysis of carboxylic acid derivatives.

A similar anomalous effect has been observed by Bruice and Bradbury[22] in the hydrolysis of mono-p--bromophenyl β-phenyl-β-substituted-glutarates. In the series of β-substituted glutaric esters under investigation they expected that any electronic effects of the substituents would influence equally the susceptibility of the ester carbonyl carbon atom to nucleophilic attack, and the nucleophilicity of the carboxylate anion. As these two effects are in opposition the net result of the electronic properties of the substituents on the rate of hydrolysis would be negligible. This assumption, however, appears to be invalid in the light of the anomalies observed in the hydrolysis of β-phenylglutaric esters. Bruice has suggested that this anomalous steric effect of the phenyl substituent may be due only in part to a polar

inductive effect. He ascribes the decrease in the expected rate of hydrolysis to be mainly due to the introduction of a site for hydrophobic bonding between the phenyl ring of the ester group and the phenyl substituent.



Such a tendency for the formation of hydrophobic bonds would decrease the population of the conformer in which the two reactant groups are eclipsed.

This explanation, however, is not applicable to the hydrolysis of α-, and β-phenylsuccinamic acids as there is no group available for hydrophobic bonding with the phenyl group.

In glutaric anhydride[82] the replacement of alkyl groups or hydrogen, in the 3-position, by a larger phenyl group appears to enhance the rate of solvolysis. This is contrary to what is observed for β-alkyl substituents, where the rate of solvolysis is decreased by the increasing bulk of the substituent. In this case Bruice accounted for the compensation of the steric

effects of the phenyl group by some polar effect which may take place by induction, or by a direct interaction of the phenyl group with the anhydride group.

The anomalous results observed for the phenyl substituents in the present study have also been attributed to their electronic properties.

Electronic Effects of the Substituents

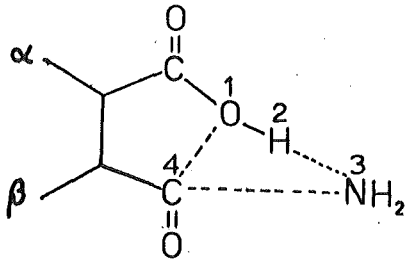
In general, the substituents that have the greatest electronic effects are electron-withdrawers (-I). Substituents which exert a positive polar effect (+I), such as alkyl groups, are invariably only weak electron--donors and their electronic effects in this case are swamped by the steric effects of the substituent. For this reason, the discussion in this section will be concerned with -I substituents. It should be noted, however, that +I substituents will give rise to similar effects, but opposite in direction to those of the -I substituents.

The electronic effects on the intramolecularly catalysed hydrolysis of succinamic acids by α- or β-substitution of -I groups are most easily considered by examining the transition states of the mechanisms concerned.

Transition States for the Hydrolysis of Succinamic Acids

Mechanism I

S_N2

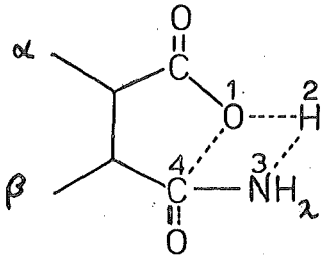


A

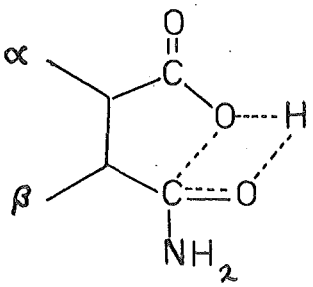
Mechanism III

Addition-elimination

B



C



The introduction of a -I substituent in the α-position will effect the electronic properties of both the carboxylic acid and the amide functions. However, its closer proximity to the carboxyl group will result in a greater interaction with this group, and its effect on the amide group will be only of secondary importance. Conversely the electronic interactions of the β substituents will be manifest mainly in the amide group.

The effect of -I substitution in the α position will weaken the bond formation (1-4) between the carboxylic

oxygen (1) and the carbonyl carbon atom (4) of the amides in all the three transition states A, B, and C, shown above.

It will also enhance the bond breaking (1-2) between the acidic proton (2) and the carboxylic oxygen (1).

In the addition-elimination mechanisms' transition states, B or C, these two effects will act in opposition, and as the importance of the protonation of the amide and the nucleophilic attack of the carboxylic oxygen will be of a similar magnitude, these two effects will tend to cancel each other.

In the transition state A of the S_N2 mechanism the breaking of the O-H bond (1-2) is only required to stabilize the leaving group and is of secondary importance to the formation of the (1-4) bond in the nucleophilic attack of the carboxylic oxygen. In this case the introduction of -I substituents in the α position would be expected to produce a substantial reduction in the rate of the intramolecularly catalysed hydrolysis.

The introduction of -I groups in the β position would be expected to increase the ease of bond formation (1-4) between the carboxylic oxygen (1) and the amide carbonyl carbon atom (4) in the three transition states A, B, and C.

For the transition states B and C the ease of protonation of the amide, on either the oxygen or the nitrogen (i.e. (2-3) bond formation), would be decreased.

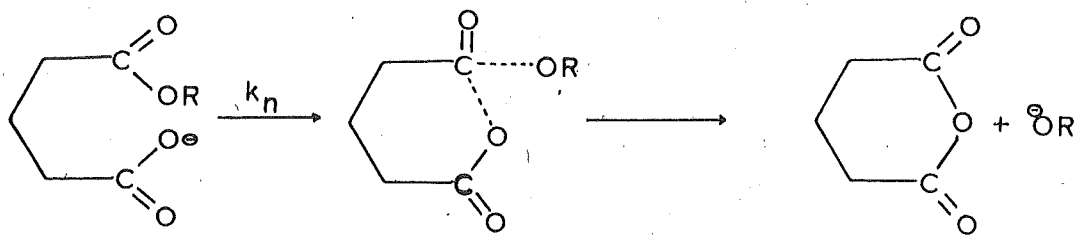
In the transition state A the breaking of the (3-4) bond between the nitrogen and the amide carbonyl carbon atom will be retarded.

The overall effect of β-substitution is, therefore, a composite one and the net effect will depend on the relative importance of the various bond formations and bond breakings involved in the transition states.

The majority of investigations of the polar effects of the substituents on the hydrolysis of carboxylic acid derivatives have involved specific hydroxide ion catalysis or specific hydronium ion catalysis. Studies of the polar effects of substituents in intramolecularly catalysed hydrolysis of carboxylic acid derivatives have been limited to the investigations of the hydrolysis of mono-p-substituted-phenyl glutarates[83] and succinates,[83] and p- and m-substituted-phenyl 4-N,N-dimethylaminobutyrates[84] and 5-N,N-dimethylamino-isovalerates.[84]

Morawetz[83] observed that the intramolecularly

catalysed hydrolysis of mono-phenyl glutarates was much faster than the corresponding intermolecular acetate ion catalysis of phenyl acetates. The intramolecular rate was also much more sensitive to the polar substituents in the phenyl group, showing a marked acceleration with -I substituents in the phenyl group. This sensitivity of the rate to the polar effects of the substituents was reflected in changes in the entropy of activation while the enthalpy of activation remained constant. A similar effect had also been observed for the ionization of the corresponding phenols[85] and on the basis of this evidence Morawetz postulated that the normal addition-elimination mechanism involving a tetrahedral intermediate was supplanted by an S_N2 mechanism.



Further support for this mechanism is demonstrated in the correlation of the logarithm of the rate constant with σ^- in the Hammett equation indicating that formation of a negative charge occurs in the leaving group in the transition state. Again, a similar correlation is observed in the data for the ionization of the

corresponding phenols.

This evidence suggests that the -I substituents increase the rate of hydrolysis by stabilizing the leaving group and have little effect on the rate of nucleophilic attack of the carboxylate anion on the carbonyl carbon atom of the ester group. It might also be expected that -I substituents in the β position of succinamic acids would have little effect on the rate of nucleophilic attack of the carboxylic oxygen on the carbonyl carbon atom of the amide. The net result of the electronic properties of -I substituents in the β position would, therefore, be a retardation in the rate of the intramolecularly catalysed hydrolysis for both mechanisms I and III.

An enhancement in the rate of hydrolysis of p- and m-substituted-phenyl esters was also observed for -I substituents by Bruice[84] in their inter- and intramolecular tertiary amine catalysed hydrolyses. The sensitivity of the rate to the polar effect of the substituent was similarly reflected in the changes of entropy of activation. No change of mechanism between the intermolecular catalysis and intramolecular catalysis was indicated by these results however, and Bruice preferred to explain them by an addition-elimination

mechanism involving a tetrahedral intermediate.

He pointed out that the difference in the mechanisms of intermolecular and intramolecular carboxylate anion catalysed hydrolysis observed by Morawetz was not one of intermolecular nucleophilic catalysis versus intramolecular nucleophilic catalysis, as originally thought by Morawetz, but one of intermolecular general base catalysis versus intramolecular nucleophilic catalysis.

Bruice[84] also stated that the electronic effects of substituents on the formation of the bond between the nucleophile and the carbonyl carbon atom of the ester would be evident in the changes in the enthalpy of activation, while the polar effects on the departure of the phenoxide ion would be expected to be most apparent in the changes in the entropy of activation. Since, in these reactions, the variations in the rates with the variation in the electronic character of the substituents in the phenyl group are only due to changes in the entropy of activation, the rate of nucleophilic attack, k_1, leading to a tetrahedral intermediate must be independent of the polar effects of the substituents.

$$\text{Substrate} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Intermediate} \xrightarrow{k_3} \text{Products}$$

$$k_I = k_1 k_3 / (k_2 + k_3)$$

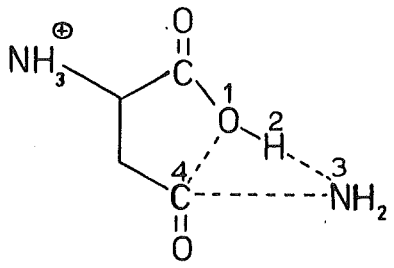
This observation, however, is more consistent with an S_N2 mechanism than with the addition-elimination mechanism preferred by Bruice, as in the latter case the overall rate, k_I, will be dominated by k_1 and only slightly affected by relatively large changes in k_3.

In the present work the polar effects of the substituents are more readily explained by an S_N2 mechanism.

The net effect of the substituents on the rate of intramolecularly catalysed hydrolysis is a composite one involving both the electronic and steric properties of the substituents.

For alkyl substituents the +I effects are small and are masked by the larger bulk effect of the substituents.

With the more highly polar substituents, methoxy-, and ammonium ion (NH_3^{+}), the polar effects predominate to produce a net retardation in the rate of intramolecularly catalysed hydrolysis. For asparagine and isoasparagine the bulk effects will be similar and the observed ten fold difference in their rates can be attributed to the relative importance of the formation of the 1-4 bond and the breaking of the 3-4 bond in the transition state A.



The importance of the nucleophilicity of the carboxylic oxygen is further demonstrated in the difference in the rates of hydrolysis of β-methoxysuccinamic acid and threo-α,β-dimethoxysuccinamic acid. In β-methoxysuccinamic acid the bulk effect of the methoxy group is cancelled by its polar effect and its rate of hydrolysis is comparable with the unsubstituted succinamic acid. The introduction of an α-methoxy group, however, produces a seven fold decrease in the rate. This decrease in the rate is even more significant if it is compared with the 250 fold difference in the rates for threo-α,β-dimethylsuccinamic acid and threo-α,β-dimethoxysuccinamic acid, where the steric effects may be regarded as similar.

If this dependance of the rates on the -I character of the α-substituents is to be adequately explained by any of the mechanisms I, II, or III the ease of formation of the bond (2-3) between the acidic proton and the amide in the transition state, must be considered, kinetically, of only secondary

importance. To account for the decrease in the rate of hydrolysis with the introduction of β -I substituents, however, mechanisms II and III require the formation of the (2-3) bond between the acidic proton and the amide to be kinetically important. The S_N2 mechanism can, therefore, be seen to be in better accord with the experimental data.

Thanassi and Bruice[17] have observed that the rate of hydrolysis of esters involving the participation of the undissociated carboxyl group showed little dependance on the electronic properties of the ester group, but for the hydrolysis of the esters involving carboxylate anion participation the rate was markedly dependent on the stability of the leaving anionic species. They attribute this to the -I effect of the ester alkoxy group favouring nucleophilic attack but disfavouring protonation of the ester. However, earlier work both by Bruice[84] and Morawetz[83] suggested that the observed differences in the relative rates of the intramolecularly catalysed hydrolysis of phenyl esters were due to the differences in the stabilities of the anionic leaving groups while the rate of nucleophilic attack was not significantly effected by the electronic character of the ester group. If a similar argument is applied to Thanassi's work the fact that only small differences

in the relative rates are observed when the hydrolysis involves the participation of an undissociated carboxyl group would imply that although the protonation of the ester group is required to stabilize the leaving group it is kinetically only of secondary importance.

The apparent anomalous bulk effect of the phenyl substituents observed in the rate of hydrolysis of α- and β-phenylsuccinamic acids may be attributed to the small -I effect of this substituent. The bulk effect predominates producing an overall acceleration of the rate of hydrolysis but its -I character is sufficient to reduce the rate of hydrolysis below that expected from the known size of the phenyl group.

The hydrolysis of α- and β-phenylsuccinamic acids exhibit an isokinetic point within the temperature range of the experimental data and, therefore, no significance can be placed on the small difference in their rates at 70°.

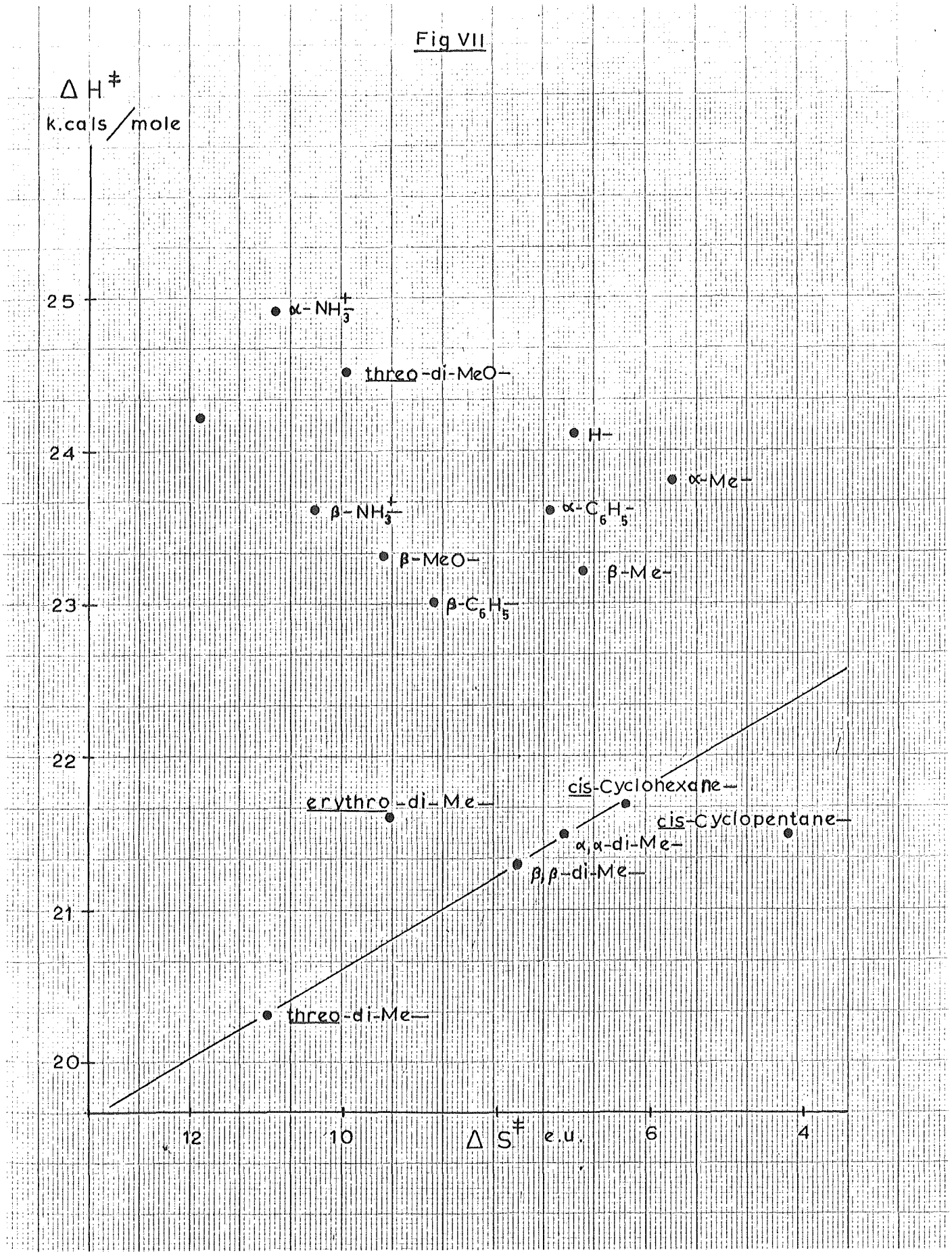
The enhanced rate of hydrolysis observed when the amino group of asparagine is acylated may be due to the composite effect of an increase in the bulk of the substituent and a decrease in its -I character. The size of the $NH_3^{\pm}$-group would be expected to be similar to that of a methyl group but solvation of the

positive charge may mean that the effective size of the protonated amino group is much larger. If such is the case, then the ten fold difference between the unimolecular rate of hydrolysis of asparagine measured in the present work and the rates of hydrolysis of the N-substituted asparagines measured by Leach and Lindley[10] is most probably a result of the change in the -I character of the substituent.

Thermodynamic Functions

The thermodynamic functions, associated with the unimolecular rate constants, in Table VIII show small random variations in the entropy of activation while the enthalpy of activation shows a steady increase with the decreasing rate constant. This is opposite to the effect observed by Bruice and by Morawetz for the intramolecularly catalyzed hydrolysis of phenyl esters, but it is in agreement with the explanation offered by Bruice.[84] He suggested that the electronic effects of substituents would show up in changes in the enthalpy of activation when the formation of the bond between the nucleophile and the carbonyl carbon atom was affected, and in changes in the entropy of activation when the stability of the leaving group was affected.

Fig VII


ΔH‡
k.cals/mole
25
24
23
22
21
20
α-NH3+
threo-di-MeO—
H—
α-Me—
β-NH3+—
α-C6H5—
β-MeO—
β-Me—
β-C6H5—
cis-Cyclohexane—
erythro-di-Me—
cis-Cyclopentane—
α,α-di-Me—
β,β-di-Me—
threo-di-Me—
12
10
ΔS‡ e.u.
6
4

A plot of $\Delta H^{\ddagger}$ vs $\Delta S^{\ddagger}$ in Fig. VII shows that there is no isokinetic relationship involving all the experimental data although one may occur for the series of cis-cyclohexane-, β,β-dimethyl-, α,α-dimethyl-, and threo-α,β-dimethyl- substituted succinamic acids. The variations of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for these compounds, however, are small and approximately equal to their probable errors.

The differences in the thermodynamic functions observed between the bimolecular rates and the unimolecular rates are also in accord with previous work[62] where the changes in the enthalpy of activation for the bimolecular reaction were found to be smaller and the entropy of activation much smaller than the corresponding functions in the unimolecular reaction.

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